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TERT promoter methylation is associated with high expression of *TERT* and poor prognosis in papillary thyroid cancer

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The telomerase reverse transcriptase (*TERT*) is overexpressed and associated with poor prognosis in papillary thyroid cancer (PTC), the most common subtype of thyroid cancer. The overexpression of *TERT* in PTC was partially attributed to transcriptional activation by two hotspot mutations in the core promoter region of this gene. As one of the major epigenetic mechanisms of gene expression regulation, DNA methylation has been proved to regulate several tumor-related genes in PTC. However, the association of *TERT* promoter DNA methylation with *TERT* expression and PTC progression is still unclear. By treating PTC cell lines with demethylating agent decitabine, we found that the *TERT* promoter methylation and the genes' expression were remarkably decreased. Consistently, PTC patients with *TERT* hypermethylation had significantly higher *TERT* expression than patients with *TERT* hypomethylation. Moreover, *TERT* hypermethylated patients showed significant higher rates of poor clinical outcomes than patients with *TERT* hypomethylation. Results from the cox regression analysis showed that the hazard ratios (HRs) of *TERT* hypermethylation for overall survival, disease-specific survival, disease-free interval (DFI) and progression-free interval (PFI) were 4.81 (95% CI, 1.61-14.41), 8.28 (95% CI, 2.14-32.13), 3.56 (95% CI, 1.24-10.17) and 3.32 (95% CI, 1.64-6.71), respectively. The HRs for DFI and PFI remained significant after adjustment for clinical risk factors. These data suggest that promoter DNA methylation upregulates *TERT* expression and associates with poor clinical outcomes of PTC, thus holds the potential to be a valuable prognostic marker for PTC risk stratification.

KEYWORDS

thyroid cancer, telomerase reverse transcriptase, methylation, prognosis, gene regulation

Introduction

Thyroid cancer is the most frequently observed malignancy of the endocrine system (1). Based on histology, thyroid cancer can be divided into four main subtypes, including papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), anaplastic thyroid cancer (ATC), and medullary thyroid cancer (MTC). Among them, PTC has a more favorable prognosis than other subtypes of thyroid cancer (2). However, as the most common subtype, PTC had a sharp increasing incidence in the past three decades and currently accounts for about 90% of all thyroid cases (3).

Overwhelming studies have demonstrated that a number of genetic alterations, including hotspot mutations and fusions in some key cancer driver genes like *BRAF*, *RAS*, *RET*, *EIF1AX* and *telomerase reverse transcriptase (TERT)*, orchestrated the initiation and progression of thyroid cancer by upregulating the expression of certain oncogenes and downregulating the expression of tumor suppressor genes (4–6). *BRAF* V600E mutation and *RET* fusions are well studied in PTC and it is now well established that they are main drivers of the aberrant MAPK pathway (7, 8). Two hotspot mutations, occurred collectively in around 10% of PTC, in the core promoter region of *TERT* activates the expression of *TERT* at the transcription level and thus promotes the tumorigenesis and development of thyroid cancer (4, 9–11). Clinically, *TERT* promoter mutations had been proved to be strongly associated with aggressive characteristics and poor prognosis of PTC (12–15).

In addition to genetic alterations, epigenetic alterations also involved in the pathogenesis of PTC. As one of the most widely studied epigenetic mechanism, DNA methylation usually observed in the promoter region of tumor suppressor genes (TSGs) and inhibits their expression in human cancers (16). The expression of several classic TSGs, including *RASSF1A*, *CDKN2A* and *DAPK*, and two thyroid-specific genes, *TSHR* and *NIS*, had been identified to be silenced by methylation (17). Interestingly, increasing studies reported that DNA hypermethylation was frequently observed in the promoter region of *TERT* in human cancers and the promoter methylation was emerged as an epigenetic mechanism of *TERT* activation (18, 19). Clinically, *TERT* hypermethylation was correlated with tumor progression and unfavorable prognosis in several types of cancer (20–23). In this study, we assessed the association of *TERT* promoter methylation with its expression and the clinical outcomes of PTC by analyzing 571 PTC samples in The Cancer Genome Atlas (TCGA) thyroid cancer database, and explored the effect of promoter DNA methylation on *TERT* expression by demethylating assays in thyroid cancer cell lines.

Methods

Clinical data

The clinical data of patients with PTC used in this study was originated from the TCGA thyroid cancer (THCA) dataset. The following clinical factors and prognostic values were collected: age at diagnosis, gender, extrathyroidal extension, pathologic T/N/M, residual tumor, pathologic stage, overall survival (OS), disease-

specific survival (DSS), disease-free interval (DFI) and progression-free interval (PFI).

TERT methylation and expression analysis

The β -value, ranging from 0 to 1, was used to show the methylation level of DNA methylation. The β -value of the CpG probe cg11625005 in the promoter of *TERT* and the Illumina HiSeq data of *TERT* expression for each thyroid sample were downloaded from TCGA dataset through the UCSC Xena platform. If the β -value of cg11625005 of one sample was higher than the mean β -value + 2*SD of the normal samples, this sample was defined as a case with *TERT* hypermethylation.

Cell line and reagents

Papillary thyroid cancer cell lines BCPAP and TPC1 were purchased from the National Collection of Authenticated Cell Cultures (Shanghai, China) and cultured in RPMI-1640 medium (#10-040-CVRC, Corning) with 10% fetal bovine serum (#04-001-1A, Biological Industries) at 37 °C. DNA demethylating drug decitabine was purchased from Selleck Chemicals (#S1200) and used for treating the two cell lines at 5 μ M for 5 days. DMSO was used as the control reagent.

Genomic DNA preparation and bisulfite sequencing PCR (BSP)

Genomic DNA was extracted from decitabine or DMSO-treated cells using the EZNA Tissue DNA Kit (#D3396-02, Omega Bio-tek) and subjected to sodium bisulfite modification using EZ DNA Methylation Lightning Kit (#D5031, Zymo Research) according to the manufacturer's instructions. The *TERT* promoter region was amplified by PCR using ZymoTaq™ PreMix (#E2004, Zymo Research) with two pairs of primers. The primer pair 5'- GGAGGAGGYGGAGTTGGAAGGTGAAGG GGTAGGA-3' and 5'- CCTCCACATCATAACCCCTCCCTCRA ATTACCCACA-3' was used for amplifying the -175 to -466 bp of *TERT* promoter, the primer pair 5'- TGTGGGGTAATyGA GGGAGGGGtATGATGTGGAGG-3' and 5'- CCTaaC TCCATTTCCACCCCTTTCTCrAC-3' was used for amplifying the -430 to -840 region of *TERT* promoter. The PCR products were then cloned to T vector and subjected to Sanger sequencing.

RNA extraction and quantitative real-time PCR

Total RNA was extracted from cultured cells using the TRIzol reagent (#15596018, Invitrogen, CA, USA) and reverse-transcribed to cDNA using the RevertAid First Strand cDNA Synthesis Kit (#K1622; ThermoFisher). Gene expression was detected in triplicate using PowerUp SYBR Green Master Mix (#A25742; Applied

Biosystem). β -Actin was used as the internal control for normalization. The primers used for *TERT* cDNA amplification were 5'-GCCTTCAAGAGCCACGTC-3' (forward) and 5'-CCACGAACTGTCGCATGT-3' (reverse), the primers for β -Actin were 5'-AGCCTCGCCTTTGCCGA-3' and 5'-GCGCGGATATCATCATC-3'. Relative mRNA expression of *TERT* was calculated according to the $2^{-\Delta\Delta CT}$ method.

Cell viability assay

Two thousand and five hundred BCPAP cells or one thousand TPC1 cells were seeded in triplicate in each well of a 96-well cell culture plate and incubated with DMSO or decitabine at concentrations ranging from 0.1 to 10 μ M for 5 days. The cell culture medium and drugs were refreshed daily. At the end of the drug-treated course, the cell viability was determined by the Cell Counting Kit-8 (#K1018, ApexBio) according to the manufacturer's instructions. The absorbance was measured at 450 nm using a spectrophotometric microplate reader.

Statistical analysis

All the *in vitro* experiments were performed three times with each done in triplicate. Comparisons of categorical variables were performed using the chi-square test. For continuous variables, the significance of differences between two groups was assessed by the Student's *t*-test. Kaplan-Meier curves with log-rank tests and Cox regression analysis were used to compare survival data by methylation status. All the statistical analyses were performed by

Stata software (version 12.0) and GraphPad Prism (version 8.0). All *P* values were 2-sided and $P < 0.05$ was considered as statistically significant.

Results

Increased *TERT* expression in PTC patients with *TERT* hypermethylation

We first compared the *TERT* methylation level in 571 thyroid cancer samples with that in 56 normal thyroid samples and found that there was no significant difference between the two groups ($P = 0.312$, Figure 1A). According to our criteria, the cut-off β -value for *TERT* hypermethylation was set at 0.494, and 33 PTC samples were defined as *TERT* hypermethylated cases. Then we analyzed the potential effect of *TERT* methylation on *TERT* expression by comparing the *TERT* mRNA levels in *TERT* hypomethylated and hypermethylated thyroid cancer samples. As shown in Figure 1B, the patients with *TERT* hypermethylation had a significant high level of *TERT* expression (1.72 ± 0.32 vs 0.40 ± 0.04 , $P < 0.001$).

Association of *TERT* methylation with clinical characteristics and outcomes of PTC

We next analyzed whether *TERT* methylation was associated with any of the following clinical characteristics of PTC: patient age, gender, extrathyroidal extension, pathologic TNM, residual tumor, and stage. The results showed that there was no significant

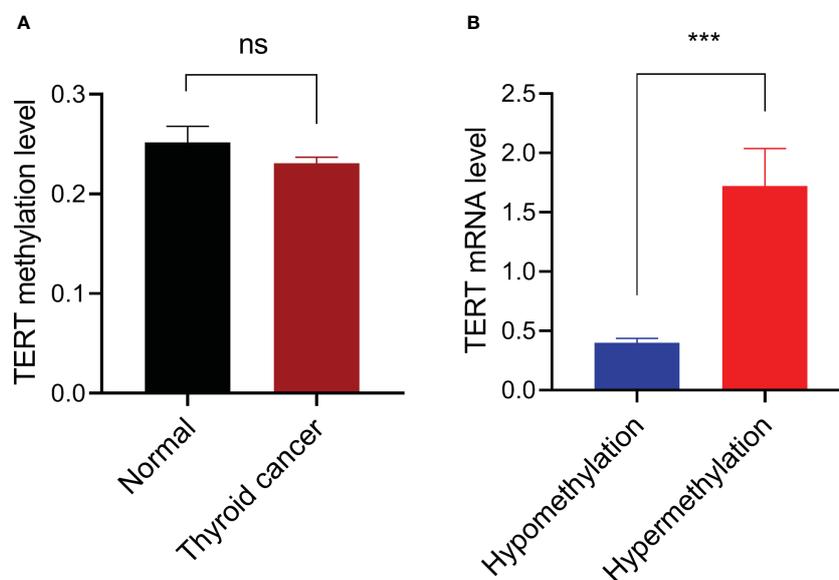


FIGURE 1

Promoter DNA methylation and *TERT* expression. (A) Comparison of *TERT* promoter methylation levels between 56 thyroid normal tissues and 571 cancer tissues. (B) Comparison of *TERT* mRNA expressions in thyroid cancer samples with *TERT* hypermethylation ($n = 33$) or hypomethylation ($n = 530$). Data were shown as mean \pm standard error. ns, not significant; *** $P < 0.001$.

correlation between *TERT* methylation and any of the clinical characteristics (Table 1). However, and importantly, patients with *TERT* hypermethylation had higher rates of poor clinical outcomes than patients with *TERT* hypomethylation (Table 2). Specifically, the overall mortality was 4 of 33 (12.1%) in *TERT* hypermethylated patients versus 16 of 538 (3.0%) in *TERT* hypomethylated patients ($P=0.023$); disease specific mortality was 3 of 32 (9.4%) in *TERT* hypermethylated patients versus 7 of 533 (1.3%) in *TERT* hypomethylated patients ($P=0.015$); tumor recurrence was 4 of 19 (21.1%) in *TERT* hypermethylated patients versus 27 of 381 (7.1%) in *TERT* hypomethylated patients ($P=0.050$); disease progression was observed in 9 of 33 (27.3%) *TERT* hypermethylated patients versus 56 of 538 (10.4%) *TERT* hypomethylated patients ($P=0.003$).

By performing Kaplan-Meier and cox regression analyses, we further assessed the impact of *TERT* methylation on the prognosis of PTC. The overall survival (OS) curves had a modest decline in patients with *TERT* hypomethylation, and declined more sharper in patients with *TERT* hypermethylation (Figure 2A). As shown in Figures 2B–D, similar patterns were obtained when analyzing disease-specific survival (DSS), disease-free interval (DFI) and progression-free interval (PFI). Results from the cox regression analysis showed that the HRs of *TERT* hypermethylation for OS and DSS were 4.81 (95% CI, 1.61–14.41) and 8.28 (95% CI, 2.14–32.13), respectively. These significances were lost after adjustment for multiple risk factors of PTC. The HRs of *TERT* hypermethylation for DFI and PFI were 3.56 (95% CI, 1.24–10.17) and 3.32 (95% CI, 1.64–6.71), respectively, and they remained

significant after multiple variables adjustment (Table 3). Then we stratified all the PTC samples into two groups according to the tumor stage, and found that *TERT* hypermethylation was associated poor clinical outcomes in advanced PTC patients (stage III/IV, Tables 2, 4 and Figure 3).

Demethylating agent treatment decreased *TERT* expression and cell viability in *TERT* hypermethylated PTC cells

To determine whether *TERT* promoter methylation regulates the gene's expression in PTC, we selected BCPAP, a PTC cell line with *TERT* hypermethylation (24), as a model and treated it with the classic demethylation agent decitabine and then checked the changes of *TERT* methylation and mRNA expression. As show in Figures 4A, B, after treatment with the demethylating agent, the methylation levels of *TERT* promoter regions -633 to -540 and -392 to -321 were decreased from 73% to 51% and from 60% to 32%, respectively. Importantly, the mRNA expression of *TERT* was significantly decreased after decitabine treatment (Figure 4C). Next, we tested whether DNA demethylation affects the growth of thyroid cancer cells and found that decitabine decreased the cell viability of BCPAP cells in a dose-dependent manner (Figure 4D). Similarly, significant *TERT* mRNA downregulation and cell growth inhibition were observed when another PTC cell line TPC1 was treated by

TABLE 1 The association of *TERT* methylation with clinical characteristics of PTC.

Characteristic	Overall	Hypermethylation		P
		Yes	No	
No.	571	33	538	
Age	46 (34–58)	51 (33–66)	46 (34–58)	0.082
Gender, male	154/571 (27.0)	12/33 (36.4)	142/538 (26.4)	0.210
Extrathyroidal extension	175/549 (31.9)	11/31 (35.5)	164/518 (31.7)	0.657
Pathologic T				
T1	155 (27.2)	8 (25.0)	147 (27.4)	
T2	191 (33.6)	9 (28.1)	182 (33.9)	0.848
T3	197 (34.6)	12 (37.5)	185 (34.4)	0.708
T4	26 (4.6)	3 (9.4)	23 (4.3)	0.197
Pathologic N	257/515 (49.9)	14/32 (43.8)	243/483 (50.3)	0.472
Pathologic M	11/332 (3.3)	1/20 (5.0)	10/312 (3.2)	0.501
Residual tumor	61/494 (12.3)	3/26 (11.5)	58/468 (12.4)	1.000
Pathologic stage				
I	324 (56.9)	16 (48.5)	308 (57.5)	
II	59 (10.4)	3 (9.1)	56 (10.4)	1.000
III	126 (22.1)	8 (24.2)	118 (22.0)	0.550
IV	60 (10.5)	6 (18.2)	54 (10.1)	0.121

TABLE 2 The association of *TERT* methylation with clinical outcomes of PTC.

Characteristic	Overall, n/N (%)	Hypermethylation, n/N (%)		P
		Yes	No	
All PTC				
Overall mortality	20/571 (3.5)	4/33 (12.1)	16/538 (3.0)	0.023
Disease specific mortality	10/565 (1.8)	3/32 (9.4)	7/533 (1.3)	0.015
Tumor recurrence	31/400 (7.8)	4/19 (21.1)	27/381 (7.1)	0.050
Disease progression	65/571 (11.4)	9/33 (27.3)	56/538 (10.4)	0.003
Stage I/II				
Overall mortality	6/383 (1.6)	0/19 (0)	6/364 (1.6)	1.000
Disease specific mortality	2/382 (0.5)	0/19 (0)	2/363 (0.6)	1.000
Tumor recurrence	20/314 (6.4)	2/14 (14.3)	18/300 (6.0)	0.221
Disease progression	29/383 (7.6)	2/19 (10.5)	27/364 (7.4)	0.646
Stage III/IV				
Overall mortality	14/186 (7.5)	4/14 (28.6)	10/172 (5.8)	0.013
Disease specific mortality	8/181 (4.4)	3/13 (23.1)	5/168 (3.0)	0.013
Tumor recurrence	11/86 (12.8)	2/5 (40.0)	9/81 (11.1)	0.121
Disease progression	36/186 (19.4)	7/14 (50.0)	29/172 (16.9)	0.007

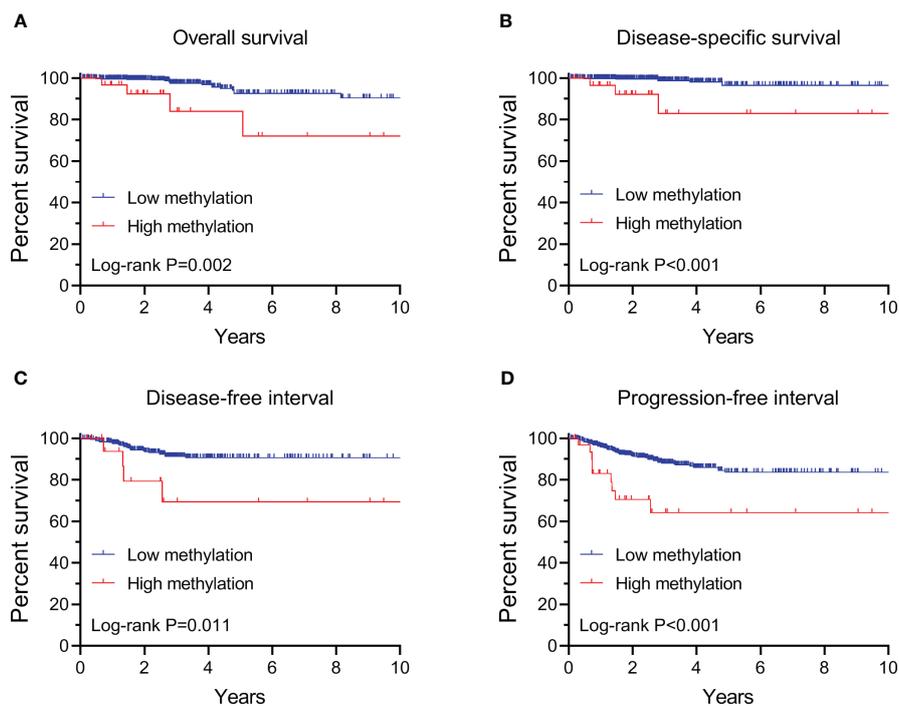


FIGURE 2 Kaplan-Meier analysis of the association of *TERT* methylation with clinical outcomes of all PTC. Results of the analysis for overall survival (A), disease-specific survival (B), disease-free interval (C) and progression-free interval (D). The P values labeled in each panel were calculated by log-rank tests.

TABLE 3 Hazard ratios of *TERT* methylation for clinical outcomes of all PTC.

	1000-person years	Crude HR (95% CI)	Adjusted HR (95% CI)*
OS			
Low methylation	8.55 (5.24-13.95)	1.00	1.00
High methylation	40.65 (15.26-108.31)	4.81 (1.60-14.41)	2.42 (0.65-9.06)
DSS			
Low methylation	3.74 (1.78-7.85)	1.00	1.00
High methylation	30.49 (9.83-94.53)	8.28 (2.14-32.13)	2.28 (0.34-15.36)
DFI			
Low methylation	20.11 (13.79-29.32)	1.00	1.00
High methylation	62.04 (23.28-165.30)	3.56 (1.24-10.17)	3.72 (1.28-10.81)
PFI			
Low methylation	32.00 (24.62-41.58)	1.00	1.00
High methylation	102.75 (53.46-197.48)	3.32 (1.64-6.71)	2.66 (1.24-5.67)

*Adjusted for age at diagnosis, sex, extrathyroidal extension, pathologic T and N. OS, overall survival; DSS, disease specific survival; DFI, disease free interval; PFI, progression free interval.

decitabine (Figures 4C, D). These data suggest that promoter DNA methylation positively regulates *TERT* expression in PTC cells and demethylation treatment suppresses the growth of PTC cells.

Discussion

TERT is regarded as a key oncogene in the tumorigenesis and development of human cancer. It is the catalytic subunit of telomerase which is essential for maintaining the length of telomere in cancer cell immortalization (25). And increasingly recent studies identified that *TERT* is also involved in cancer progression in a telomere-independent manner. For example, knockdown of *TERT* induced quick inhibition of cell proliferation, migration and invasion, while overexpression of *TERT* variants that lack telomerase activities promotes cell proliferation (26–28). In addition, *TERT* regulates NF- κ B -dependent gene expression by binding to the promoter region of these genes, suppresses TGF- β -dependent growth suppression, and function as a central regulator of various hallmark features of cancer, including resistance to apoptosis, angiogenesis, metabolism reprogramming, genome instability (29, 30).

TERT is overexpressed in thyroid cancer and associated with poor prognosis of PTC (31). Previous studies had shown that *TERT* promoter mutation is one of the major contributors to the gene's overexpression in thyroid cancer (32–34). Molecular studies further

TABLE 4 Hazard ratios of *TERT* methylation for clinical outcomes of advanced PTC.

	1000-person years	Crude HR (95% CI)	Adjusted HR (95% CI)*
OS			
Low methylation	21.67 (12.30-38.15)	1.00	1.00
High methylation	50.92 (26.49-97.85)	5.86 (1.83-18.77)	2.94 (0.75-11.49)
DSS			
Low methylation	10.83 (4.87-24.11)	1.00	1.00
High methylation	45.26 (22.63-90.50)	9.00 (2.14-37.77)	4.51 (0.63-32.29)
DFI			
Low methylation	35.00 (18.21-67.27)	1.00	1.00
High methylation	120.68 (30.18-482.54)	5.38 (1.15-25.14)	4.99 (0.94-26.55)
PFI			
Low methylation	64.50 (45.62-91.21)	1.00	1.00
High methylation	125.33 (79.94-196.48)	3.95 (1.73-9.04)	2.83 (1.10-7.33)

*Adjusted for age at diagnosis, sex, extrathyroidal extension, pathologic T and N. OS, overall survival; DSS, disease specific survival; DFI, disease free interval; PFI, progression free interval.

revealed that the two mutations in *TERT* promoter generated consensus binding sites for ETS transcription factors and thereby promoted *TERT* activation (4, 35–37). A large number of genetic association studies have demonstrated that *TERT* promoter mutation is associated with multiple characteristics of aggressive tumor behaviors and patients with *TERT* promoter mutation had a higher risk of recurrence and mortality (12–15, 38).

In this study we found that there is no significant difference of *TERT* promoter methylation level between normal thyroid tissues and PTC tissues, suggesting that promoter DNA methylation had little contribution to the overall increase of *TERT* overexpression in PTC. It should be noticed that the average level of *TERT* methylation is low in thyroid tissues, therefore we next defined hypermethylation in the tumor samples and obtained 33 of 531 (5.8%) *TERT* hypermethylated samples. Compared with patients carrying hypomethylated *TERT*, the *TERT* hypermethylated patients had significantly higher *TERT* expression. These data suggest that hypermethylation in the *TERT* promoter is likely to positively regulate *TERT* expression.

To confirm this, we assessed the effect of DNA methylation on *TERT* expression in a cell line model. After treating the PTC cells with demethylating agent decitabine, we found that the methylation level of *TERT* promoter was remarkably decreased and the expression of *TERT* mRNA was correspondingly decreased significantly. To the best of our knowledge, this is the first experimental evidence to show that *TERT* promoter DNA

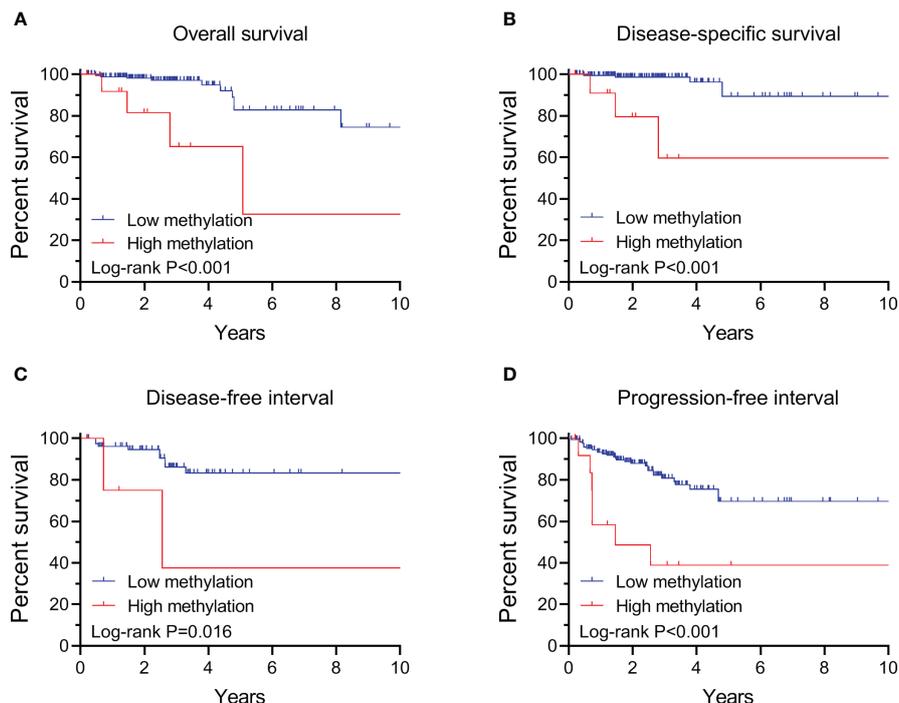


FIGURE 3 Kaplan-Meier analysis of the association of *TERT* methylation with clinical outcomes of advanced PTC (stage III/IV). Results of the analysis for overall survival (A), disease-specific survival (B), disease-free interval (C) and progression-free interval (D). The P values labeled in each panel were calculated by log-rank tests.

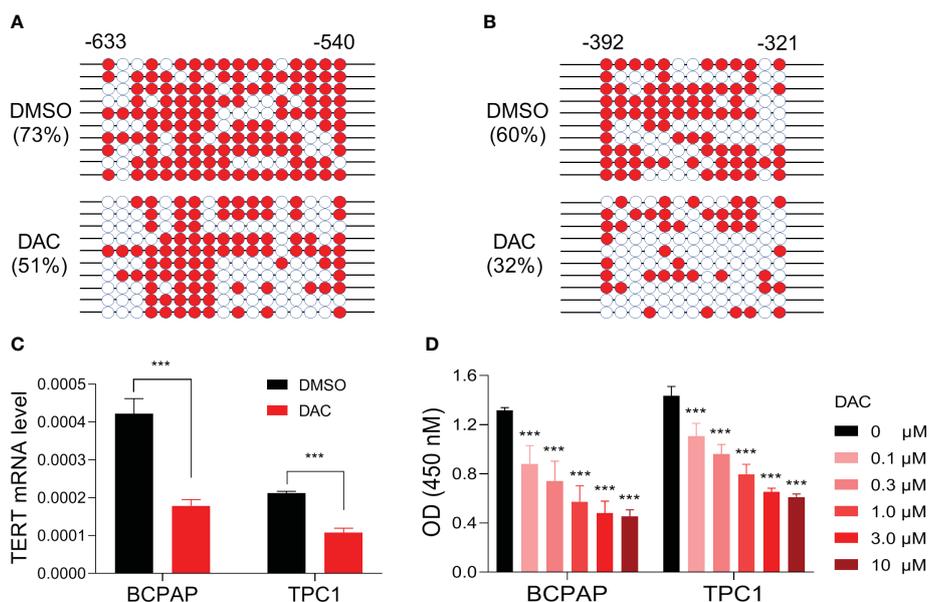


FIGURE 4 Impact of demethylating treatment on *TERT* expression and cell viability in PTC cell lines. DNA methylation levels of *TERT* promoter region regions -633 to -540 (A) and -392 to -321 (B) in BCPAP cells treated with DMSO or 5 μ M of decitabine for 5 days. The methylated clones were shown with red color, and the unmethylated clones were shown with white color. (C) *TERT* mRNA levels in PTC cell lines treated with or without decitabine. The relative mRNA expression of *TERT* was determined by qPCR. (D) The absorbances of cells treated with DMSO or decitabine at various doses as indicated. DAC, decitabine. *** $P < 0.001$.

methylation positively regulates *TERT* expression in PTC. Our finding is consistent with previous report that the DNA methylation in the *TERT* hypermethylated oncological region upregulates the promoter's activity (18). There are at least three possible mechanisms underlying the methylation mediated *TERT* activation. First, the transcriptional activator GSC showed the most remarkable binding in PTC cells than other cell lines, suggesting GSC acts as a putative regulator for methylated *TERT* in PTC (24). Second, allele-specific regulation of *TERT* was proposed to explain its activation in human cancer, and an interplay between *TERT* promoter mutation, methylation, and histone modification were involved in this process (24, 39–42). Third, a 1.6 kb antisense long non-coding RNA, named h*TERT* antisense promoter-associated (hTAPAS), in the *TERT* promoter region was proved to regulate *TERT* expression through DNA methylation in urothelial cancer (43). As the epigenetic regulation on gene expression is usually occurred in a tissue type dependent manner, whether the methylation of hTAPAS involves *TERT* regulation in PTC needs to be further pursued.

The associations between *TERT* methylation and clinical tumor behaviors and outcomes of thyroid cancer had been investigated by few preliminary studies with limited number of thyroid patients (44, 45). In this study we comprehensively analyzed the correlation between *TERT* methylation and clinical outcomes of PTC by enrolling 571 samples from the TCGA thyroid cancer database, and found that patients with *TERT* hypermethylation had unfavorable OS, DSS, DFI and PFI. Importantly, the association between *TERT* hypermethylation and DFI and PFI is still significant after adjusting multiple clinical parameters. These data strongly indicated that DNA hypermethylation in *TERT* promoter is associated with poor prognosis of PTC, and it could be serving as a prognostic marker for risk stratification of PTC. Similarly, the prognostic role of *TERT* promoter methylation had been reported in several other types of cancer, including childhood brain tumors (20), pancreatic cancer (21), bladder cancer (22), and breast cancer (23).

In conclusion, our data showed that DNA hypermethylation in the promoter region of *TERT* upregulated *TERT* expression in PTC, and *TERT* promoter hypermethylated patients with PTC had a poor clinical outcome. Our findings in this study indicate a prognostic value of *TERT* methylation in PTC and needs to be validated in further studies.

Data availability statement

The publicly available TCGA data were analyzed and the TCGA thyroid cancer datasets used in this study were available at the Xena TCGA data hub ([https://xenabrowser.net/datapages/?cohort=TCGA%20Thyroid%20Cancer%20\(THCA\)](https://xenabrowser.net/datapages/?cohort=TCGA%20Thyroid%20Cancer%20(THCA))).

Ethics statement

Ethical approval was not required for the studies on humans in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

Author contributions

SL: Methodology, Resources, Writing – original draft, Data curation, Formal analysis, Investigation, Writing – review & editing. JX: Investigation, Data curation, Formal analysis, Methodology, Writing – review & editing. KJ: Writing – review & editing, Data curation, Formal analysis, Investigation, Methodology. YC: Writing – review & editing, Data curation, Investigation, Methodology. LZ: Writing – review & editing, Data curation, Investigation, Methodology. RL: Writing – review & editing, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft.

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Conflict of interest

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The author(s) declared that they were an editorial board member of *Frontiers*, at the time of submission. This had no impact on the peer review process and the final decision.

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