EnM
ENDOCRINOLOGY AND METABOLISM

Original Article

Endocrinol Metab 2016;31:100-104
http://dx.doi.org/10.3803/EnM.2016.31.1.100
pISSN 2093-596X · eISSN 2093-5978

Low Prevalence of Somatic *TERT* Promoter Mutations in Classic Papillary Thyroid Carcinoma

Min Ji Jeon[1], Won Gu Kim[1], Soyoung Sim[2], Seonhee Lim[2], Hyemi Kwon[1], Tae Yong Kim[1], Young Kee Shong[1], Won Bae Kim[1]

[1]Department of Internal Medicine, [2]Asan Institute of Life Sciences, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Background: Transcriptional activating mutations of telomerase reverse transcriptase (*TERT*) are associated with more aggressive thyroid cancer. We evaluated the significance of *TERT* promoter mutations in Korean patients with classic papillary thyroid cancer (PTC).

Methods: Genomic DNA was isolated from four thyroid cancer cell lines and 35 fresh-frozen PTC tissues. *TERT* promoter mutations (C228T and C250T) and the *BRAF* V600E mutation were evaluated by polymerase chain reaction amplification and direct sequencing.

Results: The CC228229TT mutation in the *TERT* promoter was detected in BCPAP cells and the C250T mutation was found in 8505C cells. No *TERT* promoter mutation was observed in Cal-62 or ML-1 cells. The C228T mutation was found in only 1 of 35 (2.8%) PTCs and no C250T mutations were detected in any of the study subjects. The *BRAF* V600E mutation was found in 20 of 35 (57.1%) PTCs. One patient with the C228T *TERT* mutation also harbored the *BRAF* V600E mutation and developed a recurrence.

Conclusion: The prevalence of somatic *TERT* promoter mutations was low in Korean patients with classic PTC. Therefore, the prognostic role of *TERT* promoter mutations might be limited in this patient cohort.

Keywords: Thyroid cancer, papillary; Telomerase; Mutation

INTRODUCTION

Molecular-based risk stratification for thyroid cancer has been proposed due to recent advances in the understanding of the molecular mechanism of thyroid cancer [1]. The *BRAF* V600E mutation is the most well-known prognostic molecular marker in papillary thyroid cancer (PTC) [2,3]. The *BRAF* V600E mutation is associated with poor clinicopathological characteristics of PTC and is independently associated with frequent recurrence and higher mortality [4-6].

Previous studies have reported various prevalence rates of the *BRAF* V600E mutation in patients with PTC of 30% to 80% [2,7,8] and the prevalence of the *BRAF* V600E mutation in Korea is higher than that in other geographic regions. As a result, the prognostic value of the *BRAF* V600E mutation is limited for managing Korean patients with PTC [9-12]. Therefore, better molecular markers for predicting the prognosis of PTC are required, particularly in *BRAF* V600E-prevalent regions.

Received: 14 July 2015, **Revised**: 7 August 2015, **Accepted:** 20 October 2015
Corresponding author: Won Gu Kim
Department of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea
Tel: +82-2-3010-5883, **Fax:**+82-2-3010-6962, **E-mail:** wongukim@amc.seoul.kr

Telomerase reverse transcriptase (*TERT*) is a catalytic subunit of telomerase that plays a key role in cell immortality and tumorigenesis [13]. Frequent mutations in the *TERT* promoter region have been reported in some solid cancers, including thyroid carcinomas [14-18]. Two hotspot points in *TERT* promoter mutations are at −124 bp (C228T) and −146 bp (C250T) upstream from the ATG start codon. These mutations increase *TERT* promoter activities and expression [13]. Some studies have suggested that these mutations are associated with more aggressive thyroid cancers, such as poorly differentiated carcinoma and anaplastic thyroid cancer (ATC). Such mutations are also detected in 12% of classic PTCs and are associated with an extremely poor prognosis when accompanied by the *BRAF* V600E mutation [19].

We evaluated the significance of *TERT* promoter mutations in Korean patients with PTC. We evaluated the *TERT* promoter and *BRAF* V600E mutation status in classic PTCs having general clinicopathological characteristics using fresh-frozen tissues.

METHODS

Cell culture and reagents

The human thyroid cancer cell lines BCPAP, Cal-62, 8505C, and ML-1 were obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany) and maintained as recommended. All cell lines were authenticated by short tandem repeat profiling. HiYield Genomic DNA Mini kits were purchased from Real Biotech Corp. (Taipei, Taiwan). DNeasy Blood & Tissue kits and QIAquick polymerase chain reaction (PCR) purification kits were purchased from Qiagen (Valencia, CA, USA). The AccuPower PCR premix was purchased from Bioneer (Daejeon, Korea). Media and cell culture reagents were purchased from Gibco (Grand Island, NY, USA).

DNA extraction from thyroid cancer cells

BCPAP cells originating from PTC, Cal-62 and 8505c cells originating from ATC, and ML-1 cells originating from follicular thyroid cancer (FTC) cells, were used for DNA extraction. Genomic DNA was isolated from thyroid cancer cells using HiYield Genomic DNA Mini kits.

DNA extraction from fresh-frozen PTC tissues

Thirty-five fresh-frozen PTC tissues from surgically removed thyroid samples were collected at the Asan Bio-Resource Center and were used to isolate genomic DNA following Institutional Review Board approval (2013-0539). These tissues were selected randomly from the tissue lists at the Asan Bio-Resource Center. Genomic DNA was isolated from 10 mg fresh-frozen PTC tissue using the DNeasy Blood & Tissue kit according to the manufacturer's instructions.

Table 1. Primers Used to Amplify the *TERT* Promoter Mutation by Polymerase Chain Reaction

	Primer sequence
Sense	5'-GTCCTGCCCCTTCACCTTC-3'
Antisense	5'-TCAGCGCTGCCTGAAACTC-3'

TERT, telomerase reverse transcriptase.

PCR and sequencing

Genomic DNA extracted from thyroid cancer cells and fresh-frozen PTC tissues was amplified using PCR and sequenced to evaluate the C228T and C250T *TERT* promoter mutations. The primers are listed in Table 1. Each reaction mixture contained 10 mM Tris (pH 9.0), 250 μM of each deoxynucleotide triphosphate, 1.5 mM $MgCL_2$, 30 mM KCl, 1 μL each primer, 200 ng extracted DNA, and 1 U DNA polymerase (Bioneer) in a final volume of 20 μL. The amplification protocol consisted of initial denaturation at 94℃ for 5 minutes, 34 cycles of denaturation at 94℃ for 30 seconds, annealing at 65℃ for 30 seconds, extension at 72℃ for 30 seconds, and a final extension at 72℃ for 7 minutes. A single major PCR product was confirmed by 2% agarose gel electrophoresis, and the size of the product was 164 bp. Each PCR product was purified using a QIAquick PCR purification kit and sequenced using a DNA analyzer (PRISM 373A, Applied Biosystems, Foster City, CA, USA). The *BRAF* V600E mutation was analyzed as reported previously [9].

RESULTS

TERT promoter mutation in thyroid cancer cells

We used four different thyroid cancer cells from different origins to evaluate *TERT* promoter mutations. BCPAP cells originating from PTC presented the CC228229TT *TERT* promoter mutation (Fig. 1A) and 8505C cells originating from ATC presented the C250T and C253T *TERT* promoter mutations (Fig. 1B). However, no *TERT* promoter mutations were detected in Cal-62 from ATC or ML-1 from FTC (data not shown).

TERT promoter mutations in PTC

We evaluated *TERT* promoter mutations in PTC tissues of 35 patients. The baseline characteristics of the patients are described in Table 2. Median patient age was 45 years and 28 pa-

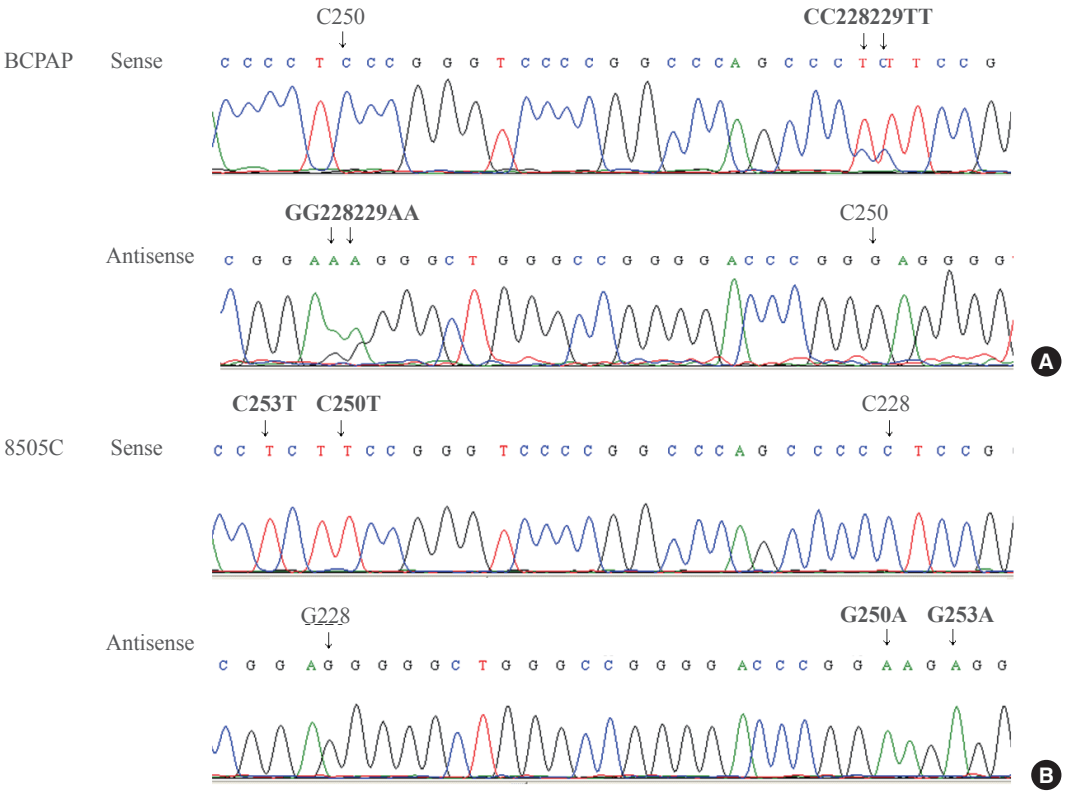

Fig. 1. Direct sequencing results of the human transcriptional activating mutations of telomerase reverse transcriptase (*TERT*) promoter in thyroid cancer cell lines. (A) BCPAP cells had the CC228229TT mutation in the *TERT* promoter. (B) 8505C cells had the C250T and C253T mutations in the *TERT* promoter.

Table 2. Baseline Patient Characteristics

Characteristic	Value
Total no.	35
Age, yr	45 (22–58)
Female sex	28 (80)
Tumor size, cm	2.2 (1.7–2.8)
Extrathyroidal extension, yes	33 (94)
Lymph node metastasis	
N0/Nx	9 (26)
N1a	22 (63)
N1b	4 (11)
Recurrence	7 (20)
Distant metastasis	1 (3)

Values are expressed as median (interquartile range) or number (%).

tients (80%) were female. Median tumor size was 2.2 cm, and most of the primary tumors had extrathyroidal extension. Cervical lymph node (LN) metastasis occurred in 26 patients (74%) including four (11%) with lateral cervical LN metastasis. During the median 5.5-year follow-up, seven patients (20%) had recurrence in the lateral cervical neck LN and one patient had distant lung metastasis.

Of the 35 PTCs, only one (2.8%) had the C228T *TERT* promoter mutation (Fig. 2A). No C250T mutation was found in any of the subjects (Fig. 2B). Twenty (57.1%) had the *BRAF* V600E mutation.

A 75-year-old female patient with the C228T *TERT* promoter mutation also harbored the *BRAF* V600E mutation and had a large primary tumor (4.2 cm) with central cervical LN metastasis. She had recurrence at the lateral cervical neck LNs and underwent reoperation. She had a high anti-thyroglobulin antibody level without structurally persistent disease at the last evaluation.

DISCUSSION

We evaluated *TERT* promoter mutational status in patients with classic PTC having general clinicopathological characteristics. Among the 35 patients, the C228T *TERT* promoter mutation was detected in only one and the prevalence of *TERT* promoter mutations was 2.8%. We also evaluated the *TERT* promoter

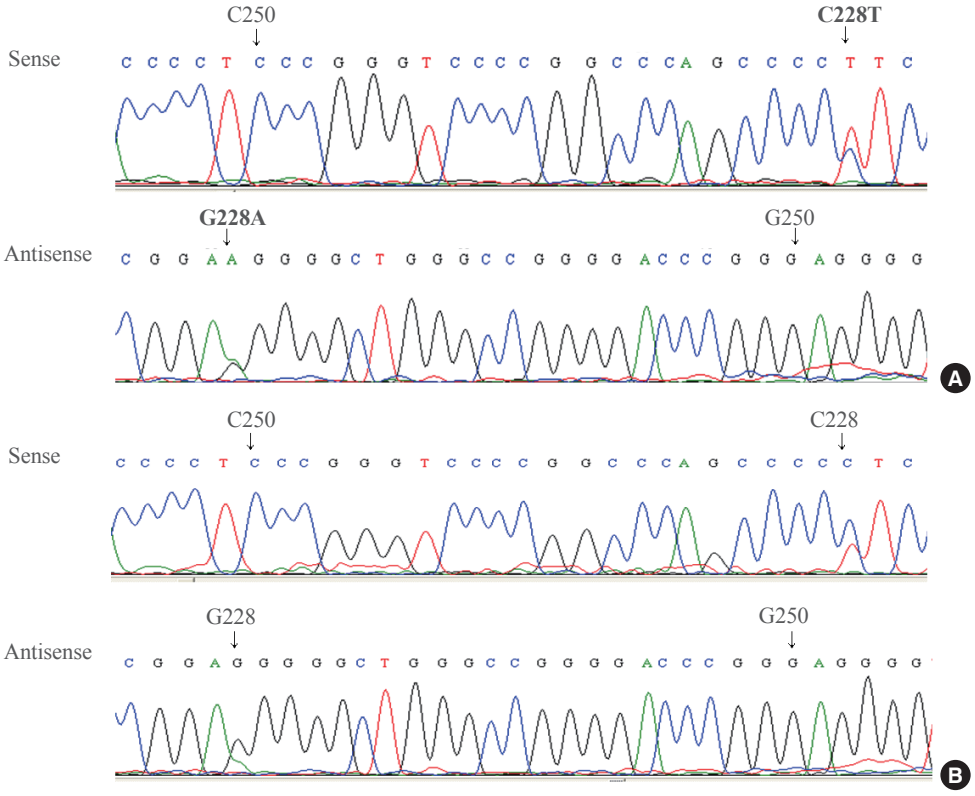

Fig. 2. Direct sequencing results of the human transcriptional activating mutations of telomerase reverse transcriptase (*TERT*) promoter in papillary thyroid cancer (PTC). (A) One patient with PTC had the C228T *TERT* promoter mutation. (B) Representative results of 34 PTCs with the wild-type *TERT* promoter.

mutation status in various thyroid cancer cell lines, and BCPAP cells from PTC and 8505C cells from ATC had *TERT* promoter mutations, consistent with a previous report [16].

Coexisting *BRAF* V600E and *TERT* promoter mutations are associated with old age, large tumor size, recurrence, and distant metastasis [19,20]. The one patient with PTC and a *TERT* promoter mutation in this study who also harbored the *BRAF* V600E mutation was old, had a large primary tumor, and developed a recurrence. She underwent a reoperation and had high serum levels of anti-thyroglobulin antibody during the follow-up.

The prevalence of *TERT* promoter mutations in patients with PTC is 8% to 17% according to previous studies from Europe and the USA [18-20]. However, we observed that only one of 35 patients (2.8%) with classic PTC had a *TERT* promoter mutation, which may have been due to the more favorable clinicopathological characteristics in our study subjects. Previous studies included more patients with progressive disease, distant metastasis, and aggressive variants of PTC [18,19]. However, differences in the genetic background of Koreans may also be a reason for the low prevalence of this mutation. Our findings suggest a limited role of *TERT* promoter mutations in Korean patients with PTC.

Our study was limited due to the small number of study subjects. However, this is the first study to present a low prevalence of *TERT* promoter mutations in Korean patients with classic PTC. Further study is required to determine the significance of *TERT* promoter mutations in Korean patients with thyroid cancer, including FTC and more aggressive thyroid cancers.

In conclusion, the prevalence of somatic *TERT* promoter mutations was low in Korean patients with classic PTC. Therefore, the prognostic role of *TERT* promoter mutations may be limited in this patient cohort.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

This study was supported by the Young Investigator's Award

(2014) from the Korean Endocrine Society. The biospecimens and data used in this study were provided by Asan Bio-Resource Center, Korea Biobank Network (2013-3(62)).

ORCID

Min Ji Jeon *http://orcid.org/0000-0002-1297-107X*
Won Gu Kim *http://orcid.org/0000-0002-8404-7759*

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