pISSN 1598-2998, eISSN 2005-9256

Cancer Res Treat. 2016;48(3):942-947

http://dx.doi.org/10.4143/crt.2015.362

Original Article

Open Access

TERT Promoter Mutations and Tumor Persistence/Recurrence in Papillary Thyroid Cancer

Jae Kyung Myung, MD[1]
Byung Kuk Kwak, MS[2]
Jung Ah Lim, MD, PhD[3]
Myung-Chul Lee, MD, PhD[4]
Min Joo Kim, MD, PhD[2]

Departments of [1]Pathology and [2]Internal Medicine, Korea Cancer Center Hospital, Seoul, [3]Department of Internal Medicine, National Medical Center, Seoul, [4]Department of Otorhinolaryngology, Korea Cancer Center Hospital, Seoul, Korea

Correspondence: Min Joo Kim, MD, PhD
Department of Internal Medicine, Korea Cancer Center Hospital, 75, Nowon-ro, Nowon-gu, Seoul 01812, Korea
Tel: 82-2-970-1215
Fax: 82-2-970-2438
E-mail: chorong24@gmail.com

Received September 24, 2015
Accepted December 17, 2015
Published Online December 28, 2015

Purpose

A telomerase reverse transcriptase (*TERT*) promoter mutation was identified in thyroid cancer. This *TERT* promoter mutation is thought to be a prognostic molecular marker, because its association with tumor aggressiveness, persistence/recurrence, and disease-specific mortality in papillary thyroid carcinoma (PTC) has been reported. In this study, we attempted to determine whether the impact of the *TERT* promoter mutation on PTC persistence/recurrence is independent of clinicopathological parameters.

Materials and Methods

Using propensity score matching, 39 patients with PTC persistence or recurrence were matched with 35 patients without persistence or recurrence, with a similar age, sex, tumor size, multifocality, bilaterality, extrathyroidal extension, and lymph node metastasis. The *TERT* promoter and the *BRAF* V600E mutations were identified from PTC samples.

Results

The *TERT* promoter mutation was detected in 18% of PTC patients (13/74). No significant difference in the frequency of the *TERT* promoter mutation was observed between the persistence/recurrence group and the non-recurrence group.

Conclusion

These results suggest that the prognostic implications of the *TERT* promoter mutation are dependent on clinicopathological parameters.

Key words
Papillary thyroid cancer, Telomerase, Thyroid neoplasms

Introduction

In cancer cells, maintenance of telomere length plays an important role in cellular stability and immortality [1]. Telomerase reverse transcriptase (*TERT*) is the catalytic subunit of telomerase, and the telomerase synthesizes repetitive DNA sequences for maintenance of telomere length [1]. Telomerase activation has been reported in several cancers including melanoma, breast cancer, and head and neck cancer [2-4].

Telomerase activation has also been identified in thyroid cancers [5]. Approximately 66% of all thyroid cancers exhibit telomerase activation [5]. However, no telomerase activation has been observed in normal thyroid tissues, suggesting that it is cancer-specific [6,7]. Therefore, telomerase activation has a potential for use as a marker of thyroid cancer.

Telomerase activation can be induced by a *TERT* promoter mutation [4]. Two hot spots of *TERT* promoter mutation in thyroid cancer cells have recently been reported [124G>A

(C228T), 146G>A (C250T)] [8-10]. The frequency of mutation differs according to the histological type of thyroid cancer. The *TERT* promoter mutation has been detected in 7%-22% of papillary thyroid carcinomas (PTCs) [9-11] and 11%-25% of follicular thyroid carcinomas [8,11,12]. However, this mutation was significantly more frequent in poorly differentiated carcinomas or anaplastic carcinomas (29%-50%) [8,11,12].

Association of the *TERT* promoter mutation with poor prognosis of thyroid cancer has been reported. The *TERT* promoter mutation was associated with older age, larger tumor size, higher stage, and distant metastasis [10-12], and with tumor persistence/recurrence and disease-specific mortality [11-13]. However, whether the association with an increased risk of tumor persistence/recurrence is independent of clinicopathological parameters remains unknown. In the current study, to assess the possible importance of the *TERT* promoter mutation on tumor persistence/recurrence, the *TERT* promoter mutation in persistence/recurrence group of patients was compared with a matched non-recurrence control group of patients.

Materials and Methods

1. Patients

This study included patients who underwent total thyroidectomy at the Korea Cancer Center Hospital between 2006 and 2012, and had PTC surgical specimens of 1.0 cm or larger. The persistence/recurrence group was selected among patients with tumor recurrence or persistence followed by a second surgery in our hospital. Persistence/recurrence was defined as the presence of a structural abnormality confirmed by surgical pathology. The non-recurrence group was defined as patients without evidence of disease or recurrence for more than 5 years after surgery. The study was approved by the Institutional Review Board of the Korea Cancer Center Hospital (K-1501-002-036), and followed the tenets of the Declaration of Helsinki.

Electronic medical records were reviewed retrospectively for collection of data on patient demographics and tumor stage, and surgical specimens were obtained for genetic analyses.

2. DNA extraction

Formalin-fixed, paraffin-embedded PTC tissues were cut into 10 μm sections. DNA was extracted using the QIAmp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

3. *TERT* promoter mutation

Polymerase chain reaction (PCR) was performed, followed by Big Dye sequencing for identification of the *TERT* promoter mutation. A 235-bp fragment containing the *TERT* promoter mutations C228T and C250T on the genomic DNA was amplified by PCR. The quality of the PCR product was confirmed by gel electrophoresis, and its sequencing was performed using a Big Dye terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems). When a mutation was found by Big Dye sequencing using the sense primer, the reaction was validated using the antisense primer.

4. *BRAF* V600E mutation

Real-time PCR was performed using the TaqMan MGB probes and FastStart Taq DNA Polymerase (Roche Life Science, Indianapolis, IN) for identification of the *BRAF* V600E mutation. Amplification and detection were performed using an ABI prism 7500 Sequence Detection System (Applied Biosystems).

5. Statistical analysis

Based on a previous study [13], we hypothesized that the frequency of the *TERT* promoter mutation was detectable in 7.5% of the non-recurrence group and 36.3% of the persistence/recurrence group, and the sample size of the study was calculated with a type 1 error (α) of 0.05 and a power (β) of 80%, resulting in 32 patients in each group.

Propensity score matching was performed to adjust for differences in the other clinicopathological features that could confound tumor persistence/recurrence. The propensity score was calculated using logistic regression with the age, sex, tumor size, multifocality, bilaterality, extrathyroidal extension (ETE), and lymph node metastasis. Thirty-nine patients with tumor persistence or recurrence were identified and 39 patients without tumor recurrence were matched using the propensity scores. Among the 39 patients from the non-recurrence group, four patients were excluded because of missing formalin-fixed, paraffin-embedded tissues or poor DNA quality, and only 35 patients were included in this study. Finally, 74 patients were analyzed. The frequencies of the *TERT* promoter and the *BRAF* V600E mutations were compared between the persistence/recurrence group and the non-recurrence group. Data are expressed as a percentage or mean±standard deviation. The independent t test or chi-square test was used when appropriate. A p-value < 0.05 was

Table 1. Clinicopathological characteristics of patients

Characterisic	Persistence/Recurrence group (n=39)	Non-recurrence group (n=35)	p-value
Age (yr)	49±13	47±14	0.64
Women	30 (77)	27 (77)	> 0.99
Tumor size (cm)	2.6±1.6	2.6±1.3	0.94
Multifocality	25 (64)	27 (77)	0.31
Bilaterality	20 (51)	21 (60)	0.36
ETE	35 (90)	31 (90)	> 0.99
LN metastasis			
Central LN	17 (44)	14 (40)	0.82
Lateral LN	22 (56)	21 (60)	
Tumor stage			
I-II	16 (41)	16 (46)	0.82
III-IV	23 (59)	19 (54)	
RAI	37 (95)	33 (94)	> 0.99
RAI dose (mCi)	208±105	199±125	0.72

Values are presented as mean±standard deviation or number (%). ETE, extrathyroidal extension; LN, lymph node; RAI, radioactive iodine; mCi, millicurie.

considered statistically significant. Statistical analyses were performed using SPSS ver. 22.0 (IBM Co., Armonk, NY).

Results

The analyses included 39 patients from the persistence/recurrence group and 35 matched patients from the non-recurrence group, using propensity scores based on age, sex, tumor size, multifocality, bilaterality, ETE, and lymph node metastasis. Patient demographics and pathological findings of surgical specimens are summarized in Table 1. The mean age of patients was 48±14 years, and 77% of the patients were female. The mean size of the primary tumor was 2.6±1.4 cm. Most patients (90%) had ETE and all patients had lymph node metastasis. No patient had distant metastasis. There were no significant differences in clinicopathological characteristics between patients from the persistence/recurrence and non-recurrence groups. Among the persistence/recurrence group, 25 patients had persistent disease and 14 recurred. Neck lymph nodes were the most common site of disease persistence/recurrence.

The time interval from the initial surgery to the identification of persistence/recurrence was 1.8±1.2 years in the persistence/recurrence group and the median follow-up time was 7.5±1.0 years in the non-recurrence group.

The *TERT* promoter mutation was detected in 18% of PTC samples (13/74). The frequency of the *TERT* promoter mutation was slightly higher in the persistence/recurrence group than in the non-recurrence group (23% vs. 12%, respectively), but there was no statistical significance (p=0.23) (Table 2). Most of the mutations (85%) presented the C228T mutation and the others presented the C250T mutation. The overall *BRAF* V600E mutation was observed in 61% of PTC samples (45/74), comprised of 54% in the persistence/recurrence group and 69% in the non-recurrence group (Table 2). No statistical difference in the *BRAF* V600E mutation was observed between patients in the persistence/recurrence and non-recurrence groups (p=0.24).

The patients were then divided according to their *TERT* promoter mutation status. As previously reported, the *TERT* promoter mutation is associated with several clinicopathological parameters (Table 3). Patients with the *TERT* promoter mutation were significantly older, had larger tumor sizes, and higher tumor stages compared to patients with the wild-type *TERT* promoter.

Discussion

In the current study, we showed that the *TERT* promoter mutation in patients from the persistence/recurrence group was not different from patients in the non-recurrence group who had similar clinicopathological characteristics. The results suggest that the *TERT* promoter mutation has no additional effect on the tumor persistence/recurrence of

Table 2. *TERT* promoter and *BRAF* V600E mutations

Mutation	Total	Persistence/Recurrence group (n=39)	Non-recurrence group (n=35)	p-value
***TERT* mutation**	13 (18)	9 (23)	4 (12)	0.23
C228T	11 (15)	8 (20)	3 (9)	
C250T	2 (3)	1 (3)	1 (3)	
***BRAF* mutation**	45 (61)	21 (54)	24 (69)	0.24

Values are presented as number (%). *TERT*, telomerase reverse transcriptase.

Table 3. *TERT* promoter mutation and clinicopathological parameters

Parameter	Mutated *TERT* (n=13)	Wild-type *TERT* (n=61)	p-value
Age (yr)	61±10	45±13	< 0.01
Women	11 (85)	46 (75)	0.72
Tumor size (cm)	3.7±2.0	2.4±1.2	0.03
Multifocality	6 (46)	46 (75)	0.04
Bilaterality	4 (31)	36 (59)	0.07
ETE	13 (100)	53 (87)	0.34
LN metastasis			
Central LN	7 (54)	24 (39)	0.37
Lateral LN	6 (46)	37 (61)	
Tumor stage			
I-II	1 (8)	31 (51)	< 0.01
III-IV	12 (92)	30 (49)	
Duration of follow-up	3.4±3.4	4.7±3.0	0.17
Persistence/Recurrence	9 (69)	30 (49)	0.23

Values are presented as mean±standard deviation or number (%). *TERT*, telomerase reverse transcriptase; ETE, extrathyroidal extension; LN, lymph node.

PTC.

Association of the *TERT* promoter mutation with tumor persistence/recurrence and the disease-specific mortality of PTC has been reported [11-13]. However, it remains uncertain whether its association is independent of clinicopathological parameters. In the current study, in comparison of patients with similar clinicopathological parameters, the *TERT* promoter mutation did not show a significant association with tumor persistence/recurrence. Xing et al. [13] reported significant association of the *TERT* promoter mutation with tumor recurrence, but there was no significance after adjustment for age, sex, multifocality, tumor size, ETE, vascular invasion, and lymph node metastasis. Although the relationship between the *TERT* promoter mutation and disease-specific mortality was significant after adjustment for age and sex [11,12], multivariate analysis for tumor size or lymph node metastasis has never been performed. Therefore, for definitive assessment of the possible impact of the *TERT* promoter mutation on the prognosis of PTC, it should be confirmed in larger studies like the *BRAF* mutation [14,15].

The *BRAF* V600E mutation also showed an association with tumor recurrence and mortality of PTC, but its association was no longer significant after the adjustment of clinicopathological parameters [14]. These results suggested that the prognostic value of the *BRAF* mutation was dependent on clinicopathological parameters.

The *TERT* promoter mutation has been reported in 7%-22% of PTC cases [9-11] and in 11% of Chinese patients with PTC [10]. The frequency of the *TERT* promoter mutation (18%) was also similar in this study. However, because the current study excluded PTC samples smaller than 1.0 cm, the overall frequency of the *TERT* promoter mutation in Korea or Asia may be lower than in Western countries.

Telomere shortening is closely associated with both cancer and aging [16]. Telomere length shortens with age [17], and a shortened telomere can activate telomerase and the *TERT* promoter mutation [12]. Consistent with our results in the current study, previous studies reported a consistent relationship between the *TERT* promoter mutation and age [10-12]. In addition, the *TERT* promoter mutation was found

more frequently in larger tumors, and there were little or no mutations in papillary thyroid microcarcinomas [10,11,18]. These results suggest that vigorous proliferation of cancer cells triggers the *TERT* promoter mutation or telomerase activation.

Past studies have reported an association of the *TERT* promoter and *BRAF* mutations [11,13]. The *BRAF* mutation is a well-known prognostic molecular marker [19], and PTCs with *BRAF* V600E mutations showed more aggressive clinicopathological characteristics [20,21]. In addition, patients with both the *TERT* C228T and *BRAF* V600E mutations were associated with higher risk clinicopathological characteristics or tumor recurrence, suggesting a synergistic effect between the two mutations [13]. However, no association was found in another study [11]. In this study, their association could not be determined because of small sample size.

Conclusion

In summary, the frequency of the *TERT* promoter mutation did not differ between patients with similar clinicopathological characteristics in the persistence/recurrence and non-recurrence groups, suggesting that the prognostic implications of the *TERT* promoter mutation were dependent on clinicopathological parameters.

Conflicts of Interest

Samkwang Medical Laboratories helped in identification of mutation.

Acknowledgments

This study was supported by a grant from the RTR (Radiological Translational Research program), Korea Institute of Radiological & Medical Sciences (50472-2015).

References

1. Smekalova EM, Shubernetskaya OS, Zvereva MI, Gromenko EV, Rubtsova MP, Dontsova OA. Telomerase RNA biosynthesis and processing. Biochemistry (Mosc). 2012;77:1120-8.
2. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, et al. Specific association of human telomerase activity with immortal cells and cancer. Science. 1994;266:2011-5.
3. Clark GM, Osborne CK, Levitt D, Wu F, Kim NW. Telomerase activity and survival of patients with node-positive breast cancer. J Natl Cancer Inst. 1997;89:1874-81.
4. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. Science. 2013;339:957-9.
5. Soares P, Lima J, Preto A, Castro P, Vinagre J, Celestino R, et al. Genetic alterations in poorly differentiated and undifferentiated thyroid carcinomas. Curr Genomics. 2011;12:609-17.
6. Matthews P, Jones CJ, Skinner J, Haughton M, de Micco C, Wynford-Thomas D. Telomerase activity and telomere length in thyroid neoplasia: biological and clinical implications. J Pathol. 2001;194:183-93.
7. Capezzone M, Cantara S, Marchisotta S, Busonero G, Formichi C, Benigni M, et al. Telomere length in neoplastic and nonneoplastic tissues of patients with familial and sporadic papillary thyroid cancer. J Clin Endocrinol Metab. 2011;96:E1852-6.
8. Liu X, Bishop J, Shan Y, Pai S, Liu D, Murugan AK, et al. Highly prevalent TERT promoter mutations in aggressive thyroid cancers. Endocr Relat Cancer. 2013;20:603-10.
9. Landa I, Ganly I, Chan TA, Mitsutake N, Matsuse M, Ibrahimpasic T, et al. Frequent somatic TERT promoter mutations in thyroid cancer: higher prevalence in advanced forms of the disease. J Clin Endocrinol Metab. 2013;98:E1562-6.
10. Liu X, Qu S, Liu R, Sheng C, Shi X, Zhu G, et al. TERT promoter mutations and their association with BRAF V600E mutation and aggressive clinicopathological characteristics of thyroid cancer. J Clin Endocrinol Metab. 2014;99:E1130-6.
11. Melo M, da Rocha AG, Vinagre J, Batista R, Peixoto J, Tavares C, et al. TERT promoter mutations are a major indicator of poor outcome in differentiated thyroid carcinomas. J Clin Endocrinol Metab. 2014;99:E754-65.
12. Liu T, Wang N, Cao J, Sofiadis A, Dinets A, Zedenius J, et al. The age- and shorter telomere-dependent TERT promoter

mutation in follicular thyroid cell-derived carcinomas. Oncogene. 2014;33:4978-84.
13. Xing M, Liu R, Liu X, Murugan AK, Zhu G, Zeiger MA, et al. BRAF V600E and TERT promoter mutations cooperatively identify the most aggressive papillary thyroid cancer with highest recurrence. J Clin Oncol. 2014;32:2718-26.
14. Xing M, Alzahrani AS, Carson KA, Viola D, Elisei R, Bendlova B, et al. Association between BRAF V600E mutation and mortality in patients with papillary thyroid cancer. JAMA. 2013; 309:1493-501.
15. Xing M, Alzahrani AS, Carson KA, Shong YK, Kim TY, Viola D, et al. Association between BRAF V600E mutation and recurrence of papillary thyroid cancer. J Clin Oncol. 2015;33: 42-50.
16. Blasco MA. Telomere length, stem cells and aging. Nat Chem Biol. 2007;3:640-9.
17. Canela A, Vera E, Klatt P, Blasco MA. High-throughput telomere length quantification by FISH and its application to human population studies. Proc Natl Acad Sci U S A. 2007;104: 5300-5.
18. de Biase D, Gandolfi G, Ragazzi M, Eszlinger M, Sancisi V, Gugnoni M, et al. TERT promoter mutations in papillary thyroid microcarcinomas. Thyroid. 2015;25:1013-9.
19. Xing M. BRAF mutation in thyroid cancer. Endocr Relat Cancer. 2005;12:245-62.
20. Xing M, Westra WH, Tufano RP, Cohen Y, Rosenbaum E, Rhoden KJ, et al. BRAF mutation predicts a poorer clinical prognosis for papillary thyroid cancer. J Clin Endocrinol Metab. 2005;90:6373-9.
21. Kim TH, Park YJ, Lim JA, Ahn HY, Lee EK, Lee YJ, et al. The association of the BRAF(V600E) mutation with prognostic factors and poor clinical outcome in papillary thyroid cancer: a meta-analysis. Cancer. 2012;118:1764-73.