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Review

BRAF and *TERT* promoter mutations: clinical application in thyroid cancer

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Abstract. Given the long-term survival of most patients with thyroid cancer, it is very important to distinguish patients who need aggressive treatment from those who do not. Conventional clinicopathological prognostic parameters could not completely predict the final outcome of each patient. Recently, molecular marker-based risk stratification of thyroid cancer has been proposed to better estimate the cancer risk. Although *BRAF* mutation has drawn much attention based on its high prevalence, its association with recurrence or mortality is not clear. Recently, telomerase reverse transcriptase (*TERT*) promoter mutation has been identified in thyroid cancer. It increases telomerase activity, which allows cancer cells to immortalize. It was found in 10 to 20% of differentiated thyroid carcinoma and 40% of dedifferentiated thyroid carcinoma. It is highly prevalent in old age, large tumor, aggressive histology, advanced stages, and distant metastasis. It is associated with increased recurrence and mortality. Concomitant *BRAF* and *TERT* promoter mutations worsen the survival rate. Inclusion of *TERT* promoter mutation analysis with conventional clinicopathological evaluation can lead to better prognostication and management for individual patients.

Key words: Thyroid neoplasms, Mutation, Recurrence, Mortality

Introduction

Papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC) referred together as differentiated thyroid carcinoma (DTC). It accounts for more than 90% in all thyroid cancers, and has a favorable prognosis [1-3]. Although the incidence of thyroid cancer has increased with time, there has been no increase in mortality rate and still remains low [4-7]. However, DTC patients who present with advanced stage or distant metastasis and patients with poorly-differentiated thyroid carcinoma (PDTC) or anaplastic thyroid carcinoma (ATC) have poor prognoses, although the number of these patients are limited [8-12]. Considering the long-term survival of most patients with DTC, it is very important to distinguish patients who need aggressive treatment from those who do not. Conventional clinicopathological parameters, such as age, histological type, tumor size, local invasion, lymph node metastasis and distant metastasis, have been traditionally used to predict the prognosis [13, 14]. However, they could not completely predict the final outcome of each patient [15]. Therefore, more precise parameters for estimating the final outcome should be required and they can make an optimal therapeutic strategy in each patient.

Two signaling pathways are involved in the development of thyroid cancer: mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinases & protein kinase B (PI3K–AKT) pathways. Both signaling pathways regulate cellular proliferation, differentiation and survival. The MAPK pathway has been known as the Ras-Raf-MEK-ERK pathway. It is frequently activated in thyroid cancer through *BRAF* mutation, *RAS* mutation, and *RET/PTC* rearrangement, which are the common initiating events in DTC [16, 17]. A few of mutations in the PI3K–AKT pathway are detected more frequently in FTC, PDTC, and ATC [18, 19].

Molecular tests for genetic alterations in thyroid cancer may enhance the diagnostic value of cytologic examination and predictability of clinical outcomes [20]. Molecular changes precede histological changes. Gupta *et al.* described heterogeneous histological changes in a tumor: microfollicular areas with well-developed nuclear features of PTC, large follicles with borderline nuclear features of PTC, and large follicles lacking nuclear

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features of PTC. When tested separately, all three areas were positive for the same *NRAS*61 mutation [21]. Much progress has been made in elucidating the molecular mechanisms involved in the pathogenesis of thyroid cancer after the detection of *RAS* mutation and *RET/PTC* rearrangement. Newly identified oncogenes have provided the basic information upon which new diagnostic tools, new prognostic markers, and new targeted agents have been developed. The Cancer Genome Atlas (TCGA) study presented integrated genomic characterization of PTC in 2014 [22]. It described the low frequency of somatic alterations in 496 PTCs compared to other cancers. It reduced the fraction of PTC cases with unknown oncogenic driver from 25% to 3.5%. Genetic alterations in thyroid cancer included point mutations (75%), gene fusions (15%), and copy number variations (7%). In patients with advanced stage, tumors may have more than one mutation, therefore, the overall mutation burden exceeds 100%. The frequency of the main drivers (*BRAF*, *RAS*, and *RET*) sums to less than 100% because in some cases the drivers are not known or they are lower frequency events [23].

In this article, I would like to update a review article published in 2018 by presenting the findings of some additional studies that have been published subsequently [24].

BRAF Mutation in Thyroid Cancer

BRAF mutations were firstly detected in human cancer in 2002 [25]. Thereafter, more than 40 mutations of *BRAF* gene have been identified. Most cases activating *BRAF* mutation involve codon 600 and result in the V600E mutation, and a few cases of other *BRAF* mutations occur as K601E mutation, small in-frame insertions, deletions, or rearrangement [16, 26]. The *BRAF* mutations were frequently detected in thyroid cancer [16, 17, 27-30]. They have been reported in PTC and PDTC or ATC arising from PTC, but not in FTC, medullary thyroid cancer (MTC) and benign thyroid tumor [31, 32]. Exceptionally, there was a report that one case with FTC carried a $BRAF^{K601E}$ mutation [33]. The thymine-to-adenine transversion at nucleotide position 1799 in exon 15 of the *BRAF* gene results in a valine-to-glutamate substitution at residue 600, which leads to constitutive activation of MAPK signaling downstream resulting in tumor development [17, 25, 34]. Small PTCs, less than 1 cm in diameter, have also been shown to harbor *BRAF* mutations, which are considered to be the early stage of PTCs or an inducing factor of oncogenesis [35-37]. The prevalence of *BRAF* mutation in PTC ranges from 30% to more than 80%, depending on the iodine intake and geographic location [36, 38]. *BRAF* mutation is known to be highly specific to PTC, but false-positive results have been rarely reported [39]. In Korea, approximately 97% of newly diagnosed thyroid cancer is PTC and more than 80% of PTC cases harbor *BRAF* mutation [3, 40, 41]. Therefore, molecular testing for the *BRAF* mutation in cytological specimen increases diagnostic sensitivity & accuracy in a *BRAF* mutation-prevalent population [40, 41].

Molecular marker-based risk stratification of thyroid cancer has been recently proposed for estimation of cancer risk [42]. The *BRAF* mutation has drawn much attention based on its high prevalence [40, 41, 43, 44]. Many studies have demonstrated that *BRAF* mutation is significantly associated with the aggressive clinicopathological characteristics of PTC, such as extrathyroidal extension, lymph node metastasis and advanced stages [29, 31, 44, 45]. Lee *et al.* performed a meta-analysis from 12 studies including 1,168 PTC patients [46]. They reported that *BRAF* mutation was associated with histologic subtype, the presence of extrathyroidal extension, and higher clinical stage, but not with age, sex, race, or tumor size. Tufano *et al.* published a meta-analysis from 14 studies including 2,470 PTC patients [47]. They demonstrated that *BRAF* mutation in PTC was significantly associated with the recurrence, lymph node metastasis, extrathyroidal extension, and advanced stages. Even in Korea, the same results were initially published with the meta-analysis of Tufano *et al.* [44, 45]. Xing *et al.* reported the association of *BRAF* mutation with high recurrence and mortality rate in a large multicenter study [48, 49]. Recently, Shen *et al.* also reported that there was a linear association between patient age and mortality in patients with $BRAF^{V600E}$ mutation, but not in patients with wild-type *BRAF* [50]. They concluded that age was a strong, continuous, and independent mortality risk factor in patients with $BRAF^{V600E}$ mutation. However, it was no longer significant after the adjustment of risk factors, such as lymph node metastasis, extrathyroidal invasion and distant metastasis. In addition, many studies from East Asian countries including Taiwan, Korea, and Japan demonstrated that the *BRAF* mutation was not associated with disease-free survival as well as poor prognostic factors [51-53]. Many physicians wonder why thyroid cancer-related mortality is still low, although the *BRAF* mutation is highly prevalent. These findings suggest that the isolated *BRAF* mutation may be a sensitive, but not specific marker of tumor recurrence and tumor-related mortality. Recently, more specific markers predictive of aggressive behavior have emerged. In patients with advanced stage, tumors may have more than one mutation. Next generation sequencing-based analysis revealed that some PTCs had more than one mutation, and these have aggressive behavior [54].

TERT Promoter Mutation in Thyroid Cancer

Telomeres are located at the ends of linear chromosomes. They are composed of hundreds to thousands of tandem DNA repeat sequences: hexameric *TTAGGG* in the leading strand and *CCCTAA* in the lagging strand in humans [55]. They were coated by protective proteins termed shelterin [56]. The 3' end of the telomeric leading strand terminates as a single-stranded overhang, which folds back and invades the double-stranded telomeric helix, forming the T loop. Telomeres can be directly visualized under the microscope at the ends of metaphase chromosomes by fluorescence *in situ* hybridization (FISH). Average telomere length can be measured by several methods: a technique that combines flow cytometry and FISH, Southern blotting, and a quantitative polymerase-chain-reaction assay. The average length of telomeres in human leukocytes varies, ranging from approximately 11 kb at birth (in umbilical-cord blood) to 6 kb at 90 years of age. Telomere loss is most rapid early in life, and over a life span [57]. The normal cell will divide 50 to 70 times before cell death (Hayflick limit). As the cell divides, the telomeres get smaller. Because of the telomeres shortening through each division, the telomeres will eventually no longer be present on the chromosome. The cells finally become senescent and cell division stops. Shortened telomeres impair immune function that might also increase cancer susceptibility [58]. Therefore, telomere shortening can be related to the risk of cancer. Telomerase is a reverse transcriptase that utilizes an RNA template to add telomeric repeats to the 3' ends of chromosomes. Active telomerase enzyme complex is composed solely of two protein components, telomerase reverse transcriptase (*TERT*) and dyskerin, and the telomerase RNA component. It protects the telomere repeats from erosion, such as DNA damage or fusion with neighboring chromosomes. Therefore, it reverses telomere shortening. Telomerase is not expressed in normal cells, but is frequently activated in most cancer cells as well as stem cells [59, 60].

Cancer cells are immortal because activated telomerase allows them to survive much longer than normal cell. Therefore, maintenance of telomere length is very important for immortalization of cancer cells. In 2013, point mutations of *TERT* promoter were firstly found in melanoma, and these somatic mutations enhanced promoter activity by two- to four-fold, which could immortalize cancer cells by maintaining telomere length [61-63]. Somatic mutations in the *TERT* promoter have been identified in over 50 human cancers including thyroid cancer [64]. *TERT* promoter mutation has been shown to increase telomerase activity, which protects the telomere repeats from erosion and maintain telomere length. Two mutations in the *TERT* promoter (chr5: 1295228C>T, termed C228T, and chr5: 1295250C>T, termed C250T) were found in melanoma [61, 62].

The C228T and C250T mutations were detected in follicular cell-derived thyroid cancer but were absent in benign tumors and MTC [64-68]. These two mutations occurred in a mutually exclusive manner. C228T is far more dominant than C250T. The mutual exclusivity of the two mutations suggests that either may function sufficiently to play an important role in thyroid tumorigenesis although which one is more powerful oncogenically has not been established [66, 69, 70]. In meta-analysis, *TERT* promoter mutation was found in approximately 10% of PTC, 17% of FTC, and 40% of PDTC/ATC [70, 71]. Among PTC, it was more prevalent in tall cell variant than conventional or follicular variants. The *TERT* promoter mutation has a significantly higher prevalence in old age, large tumor, aggressive histology, advanced stages, and distant metastasis [70, 71]. Why it is more prevalent in old age? Thyroid epithelial cells from young age are telomerase proficient with longer telomere, whereas those from later are telomerase deficient with shorter telomere. When it is attacked by oncogenic events, it undergoes active proliferation with further erosion of its telomere. In that case, telomere dysfunction or even telomere crisis occurs in old age because of its initial shorter telomere and deficient telomerase. Telomere crisis triggers genomic instability and finally induces telomerase activation. *TERT* promoter mutation is thus the consequence of genomic instability, whereas in turn contributes to derepressing *TERT* transcription and telomerase activation [67]. *TERT* promoter mutation has not been found in childhood thyroid cancer, except one in Saudi Arabia [72-75]. *TERT* promoter mutations were rarely found in small-sized thyroid cancer. de Biase *et al.* reported that *TERT* promoter mutations were found in 4.7% of the analyzed 431 papillary thyroid microcarcinomas [76]. Yang *et al.* suggested that *TERT* promoter mutation closely associated with non-radioiodine avidity in distant metastatic DTC [77]. *BRAF* mutation has been known to be associated with iodine intake, but *TERT* promoter mutation is not [78]. In a multivariate comparison between the PTC with and without anaplastic transformation, *TERT* promoter mutation was independently associated with anaplastic transformation [79]. Collectively, PTC-derived ATCs are characterized by *BRAF* and *TERT* promoter mutations, and these mutations occur prior to anaplastic transformation. Of note, a PTC harboring *TERT* promoter mutation is at higher risk for anaplastic transformation. Interestingly, *TERT* mutations in some studies were found to be more common in tumors with *BRAF* mutation, which may suggest a

possible synergistic interplay between MAPK pathway activation and telomerase activation to promote aggressive tumor behavior [65, 66]. *TERT* promoter mutation as well as *BRAF* mutation is known to be associated with tumor recurrence, but second recurrence rate is significantly high in patients with *TERT* promoter mutation, not *BRAF* mutation [80]. In a meta-analysis including 11 studies with 3,911 patients, PTC with concurrent *BRAF* and *TERT* promoter mutations were associated with increased tumor aggressiveness and had worst prognosis in comparison with PTCs harboring *BRAF* or *TERT* promoter mutation alone [81]. Recently, Song *et al.* suggested the potential mechanism of synergistic effect of *BRAF* and *TERT* promoter mutations on cancer progression [82]. They explained that *BRAF* mutation activated MAPK pathway, and it upregulated E-twenty six (ETS) transcription factors. ETS factors bound to mutant *TERT* promoter, and it increased *TERT* promoter expression. The genotype of primary tumors has high concordance with the genotype of lymph node metastasis. However, distant metastases show enrichment in *TERT* promoter mutations and a decrease in *BRAF* mutations. Therefore, *TERT* promoter mutations may play a role in distant metastases [83]. The *TERT* promoter mutation is also associated with increased mortality in PTC as well as other thyroid cancer. Liu *et al.* reported that PTC with concurrent *BRAF* and *TERT* promoter mutations were associated with increased cancer-specific mortality in comparison with PTCs harboring *BRAF* or *TERT* promoter mutation alone (*TERT/BRAF* 22.7% *vs. TERT* 6.3% *vs. BRAF* 2.4% *vs.* wild type 0.6%) [84]. Kim *et al.* evaluated the association of *TERT* promoter mutation with survival of 409 thyroid cancer patients followed for median 13 years [71]. They reported that the *TERT* promoter mutation was independently associated with poorer overall survival in patients with DTC (10-year survival, *TERT* promoter mutation 66% *vs.* wild type 98%) and in patients with PTC (74% *vs.* 99%). Concomitant *BRAF* and *TERT* promoter mutations worsened the survival rate of patients with PTC (10-year survival, both mutations 83% *vs.* wild type 99%; HR 5.62). Recently, Liu *et al.* reported that coexisting *BRAF* and *TERT* promoter mutations are strongly associated with the loss of RAI avidity in recurrent PTC, showing a robust predictive value for failure of RAI treatment of PTC [85]. Thereafter, several studies have supported the synergistic effects of concomitant *BRAF* and *TERT* promoter mutations [78, 86-88].

Clinical Application of *BRAF* and *TERT* Promoter Mutations in Thyroid Cancer

A few of studies investigated the association of *BRAF* and *TERT* promoter mutations with ultrasonography (USG) findings. Hahn *et al.* suggested that PTC with no mutation, with *BRAF* mutation alone, and with both *BRAF* and *TERT* promoter mutations linearly increased in the probability of displaying malignant USG features [89]. They also reported that PTC with *BRAF* mutation tended to show a nonparallel orientation (taller-than-wide) shape, but this finding was marginally significant (p = 0.055). There were no significant differences in tumor echogenicity, tumor margin, and calcification between *BRAF* mutation and wild type. Kim *et al.* reported that nonparallel orientation and microlobulated margin were independent USG findings for predicting *TERT* promoter mutation in PTC, especially patients over 50 years [90]. Therefore, they suggested that tests for *TERT* promoter mutation should be done when physicians met the unique USG findings (nonparallel orientation and microlobulated margin) in thyroid nodular patients, especially over 50 years.

Wang *et al.* reported an interesting observation in 2014 [91]. They identified *TERT* promoter mutation (C228T) in 1 of 58 follicular adenoma postoperatively. The patient with C228T mutated follicular adenoma later developed a scar recurrence and died of FTC, whereas none of the remaining 57 patients with follicular adenoma had recurrence. Therefore, they concluded that *TERT* promoter mutation might occur as an early genetic event in thyroid follicular tumors that have not developed malignant features on routine histopathological workup. According to this finding, it is advisable to develop a more aggressive treatment strategy when a physician sees a patient diagnosed with follicular neoplasm after histological examination but has a *TERT* promoter mutation. In my personal opinion, at least total thyroidectomy should be done in this patient with *TERT* promoter mutation, even though they were diagnosed with follicular neoplasm by histologic examination.

Xu *et al.* also reported an interesting finding in 2017 [92]. They observed 15 cases with low-risk histology with distant metastases at present. The majority was encapsulated follicular variant of PTC with capsular invasion only. Among 8 tumors that were subjected to next-generation sequencing analysis, *TERT* promoter mutation occurred at a higher rate than that seen in PTC in general and may help explain their aggressive behavior. DTC with low-risk histologic features and distant metastasis was a rare occurrence, accounting for less than 3% of metastatic non-ATC. When meet DTC cases with low-risk histology together with distant metastasis, distant metastasis is almost always found at presentation. These tumors might have a high probability to have *TERT* promoter mutations.

In 2015, the American Thyroid Association introduced

new therapeutic guidelines for DTC included the reclassification of cancer recurrence risk after initial treatment [93]. The new dynamic risk stratification (DRS) system can predict the structural recurrence with higher accuracy than traditional predictive system based on clinicopathologic information [94]. Kim *et al.* aimed to refine risk prediction for structural recurrence and cancer-specific mortality using the *TERT* promoter mutation in 357 DTC patients without initial distant metastasis with long-term follow-up (median 14 years) [95]. They developed a new integrative prognostic system that incorporates *TERT* promoter mutation into the recently proposed DRS system after initial therapy to better categorize and predict outcomes. Cox regression was used to calculate adjusted hazard ratios (AHRs) to derive AHR groups. AHR was adjusted by age, sex, histologic type, multifocality, size, extrathyroidal invasion, node metastasis & RAI treatment. They compared AHR group with pre-existing DRS system and TNM classification using proportion of variance explained (PVE). Larger numbers of PVE suggest better predictability. Patients in higher AHR groups were significantly at higher risk of recurrence and cancer-related death. They suggested that the PVE of new AHR system to predict recurrence was higher than the pre-existing DRS system (22.4% *vs.* 18.5%). PVE of new AHR system to predict cancer-related death was also higher than pre-existing TNM system (11.5% *vs.* 7.4%). Therefore, they concluded that inclusion of *TERT* promoter mutation analysis with conventional clinicopathological evaluation could lead to better prognostication and management for individual patients.

Conclusion

Although the *BRAF* mutation has drawn much attention, it cannot predict the clinical outcome of each patient. It was no longer significant after the adjustment with other prognostic factors. The isolated *BRAF* mutation may be a sensitive, but not specific marker of recurrence and mortality. *TERT* promoter mutation has been found in 10–20% of DTC and 40% of dedifferentiated thyroid cancer. Although it is highly prevalent in old age, large tumor, aggressive histology, advanced stages, and distant metastasis, it is strongly associated with tumor recurrence and mortality in thyroid cancer. Therefore, inclusion of *TERT* promoter mutation analysis with conventional clinicopathological evaluation could lead to better prognostication and management for individual patients.

Conflicts of Interest

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

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