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Full Title: TERT promoter mutation predicts radioiodine refractory in distant metastatic differentiated thyroid cancer

Short Title: TERT mutation predicts iodine-refractory

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ABSTRACT

Telomerase Reverse Transcriptase (TERT) promoter mutation has been reported to be associated with aggressive characteristics in differentiated thyroid cancer (DTC). This study examined the status of TERT promoter mutation in distant metastatic DTC (DM-DTC), and evaluated the correlation between TERT mutation and radioactive iodine-131(RAI) uptake, as well as that between TERT mutation and therapy response.

Methods: TERT promoter and B-Raf proto-oncogene (BRAF) V600E mutation were retrospectively examined in primary tumors of 66 DM-DTC patients. Stimulated thyroglobulin (sTg) changes, RAI uptake status (avid or non-avid), and other imaging evidence were analyzed to evaluate therapy response. After a median follow-up of 46.5 months (interquartile range, 29.0 to 70.5 months), therapy response was classified as disease control and refractory.

Results: The prevalence of TERT mutations was 22.73% (15/66), of which C228T mutation was more prevalent (13/15) than C250T mutation (2/15). Rising sTg was noticed in 93.33% (14/15) of TERT mutation group. While in cases with both mutations negative, 78.12 % (25/32) presented with decreased sTg. TERT mutation closely correlated with poor RAI therapy response ($p<0.001$), and all 15 patients were classified as refractory to RAI with a positive predictive value of 100% at the end point of follow-up. TERT mutation was associated with older mean age at diagnosis ($p<0.001$), larger mean tumor diameter ($p=0.013$), and more likelihood of both BRAF mutation coexistence ($p=0.044$) and refractory to RAI ($p<0.001$). In the 36 cases received imaging semi-quantitative analysis, it was found that TERT mutation significantly correlated with non-RAI-avidity, with a much lower mean tumor/background (T/B)

ratio (obtained from post RAI therapy whole-body scanning) than TERT wild-type ($p<0.001$). And DM-DTC patients with TERT mutation were more likely to lose RAI-avidity at initial RAI therapy than those with only BRAF mutation (8/8 vs 5/11, Fisher's exact test, $p=0.018$).

Conclusions: TERT promoter mutation closely associates with non-RAI-avidity in DM-DTC, and when comparing with BRAF mutation, TERT mutation manifested a worse negative influence on RAI uptake. It could also be used as a predictive marker to early identify refractory to RAI.

Key words: Differentiated thyroid carcinoma; TERT promoter mutation; Radioactive iodine therapy; Therapy response

INTRODUCTION

Although differentiated thyroid cancer (DTC) presents a favorable clinical outcome with the 10-year overall survival rate around 90%, it can be life-threatening when distant metastasis occurs. Distant metastasis can be observed in 2.2%-23% DTC patients (*1*,*2*). Serial treatments with radioactive iodine (RAI) can render 10-year survival rate of 42%, despite of the fact that distant metastasis contributes most to the mortality of DTC (*3*). The efficacy of RAI therapy largely depends on the ability of RAI uptake in distant metastatic lesions, and 10-year survival rate can be as high as 56% in patients with intense RAI accumulation, while as low as 10% in those who lose RAI avidity (*3*). Therefore, the RAI concentrate pattern closely correlated with RAI therapy response and clinical outcome. Early identification of non-RAI avidity would be helpful in avoiding unnecessary RAI therapy and timely adjusting subsequent feasible therapy schedule in those RAI refractory patients. In order to early recognize the status of RAI refractory, ^{99m}Tc-3PRGD2 imaging targeting integrin α v β 3 was used to trace RAI refractory metastatic lesions in our previous study, which could detect the active angiogenesis of RAI refractory foci with rapid growth (*4*). Another study of our group indicated that B-Raf proto-oncogene (BRAF) V600E mutation associated with non-RAI-avid status of distant metastatic DTC (DM-DTC) (*5*), implying that the molecular characteristics may drive the early identification of non-RAI-avidity. More recently, Telomerase Reverse Transcriptase (TERT) promoter mutation has been demonstrated as one of aggressive molecular markers for follicular cell-derived thyroid neoplasm (*6*). Two prevalent hotspots of C228T and C250T could cause hyperactivity of TERT promoter, and induce over proliferation and carcinogenesis (*7*,*8*). To date, it remains unclear whether TERT

mutation is associated with RAI uptake status in distant metastatic lesions and RAI therapy response.

The aim of the present study was to report the prevalence and frequency of TERT promoter mutation in Chinese DM-DTC patients, and analyze the correlation of TERT promoter mutation with RAI uptake status and RAI therapy response in distant metastatic lesions.

MATERIALS AND METHODS

Patients and tissue samples

The study protocol was approved by the ethical board of Chinese Academy of Medical Sciences and Peking Union Medical College Hospital, and all subjects signed a written informed consent.

Sixty-six cases of DM-DTC were retrospectively retrieved from Peking Union Medical College Hospital. The overall median follow-up time was 46.5 months (interquartile range, 29.0 months to 70.5 months) after initial treatments. All patients were referred for at least two courses of RAI therapy after total thyroidectomy from August 2008 to January 2016, among which 36 patients underwent both surgeries and each course of subsequent RAI therapy in Peking Union Medical College Hospital, whose intact data of both treatments and follow-up allow us for further imaging semi-quantitative analysis.

Imaging analysis

Distant metastases were identified in terms of imaging examinations such as chest computerized tomography, 18-fluorodeoxyglucose positron emission tomography/computerized tomography, bone scan, diagnostic radioiodine whole-body scanning, and post radioiodine

therapy whole-body scanning, in combination with elevated thyroglobulin level.

Semi-quantitative analysis of RAI uptake in metastatic foci was conducted by the same clinician using single-photon emission computed tomography regions of interest software. Briefly, the mean counts of regions of interest target lesions (T) and background (B) were drawn on the target lesions and normal frontal cranial bone separately, and T/B ratios were obtained. In order to avoid the interference of thyroid residue, we calculated two T/B ratios for 36 patients, one after the first RAI therapy with thyroid residue (T/B1), and the other after the time of RAI therapy with residual thyroid tissue completely ablated (T/B2). According to the findings on whole-body scanning in distant metastatic lesions, RAI uptake status was defined as RAI-avid or non-RAI-avid.

Serological examination

Serological examinations, including thyroid stimulating hormone, thyroglobulin and anti-thyroglobulin antibody (TgAb) levels, were measured before RAI therapy and during the follow-up. Patients with positive TgAb level were ruled out. Thyroglobulin and TgAb levels were measured using an electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany), and the thyroid stimulating hormone level was measured using a chemiluminescence immunoassay (Siemens Healthcare Diagnostics Inc., New York, NY, USA) in the same laboratory. Thyroglobulin assay had a functional sensitivity of 0.1 ng/ml. Stimulated thyroglobulin (sTg) was used as a serological marker to evaluate response to RAI therapy. After at least two courses of RAI therapy, serological responses were defined according to the changes in sTg level into three types: ⅰ) Up: at least 20% increase compared with basal sTg which

measured at the time of the first RAI therapy; ⅱ) Down: at least 20% decrease than basal sTg; ⅲ) Stable: sTg fluctuation from -20% to 20%. At the end point of follow-up period, RAI therapy responses were classified as: ⅰ) disease control and ⅱ) refractory.

Evaluation criteria for RAI refractory DTC

Radioiodine-refractory structurally-evident DTC is defined in patients with appropriate thyroid stimulating hormone stimulation and iodine preparation in four basic ways based upon 2015 American Thyroid Association management guidelines (*9*), in combination with 2014 Chinese management guidelines on DTC treatment by RAI (*10*) : ⅰ) the metastatic tissue does not concentrate radioiodine even after successful remnant thyroid ablation; ⅱ) the tumor tissue loses the ability to concentrate radioiodine after previous evidence of RAI-avid disease (in the absence of stable iodine contamination); ⅲ) radioiodine is concentrated in some lesions but not in others; ⅳ) metastatic disease progresses despite significant concentration of radioiodine.

DNA isolation

Genomic DNA was extracted from four 5 μm-thick slices of formalin-fixed paraffin-embedded DTC primary tumor samples, using a commercial DNA extraction kit (GeneRead DNA formalin-fixed paraffin-embedded kit, Qiagen, Catalog No. 180134, Germany) according to the manufacturer's instructions. The tumor samples were manually dissected under microscopic guidance by an empirical pathologist of Peking Union Medical College Hospital and any adjacent normal tissue surrounding the tumor was pared away.

TERT promoter mutation analysis

The TERT promoter mutations were analyzed by Polymerase Chain Reaction (PCR)

amplification and direct Sanger sequencing of the hot mutation position of C228T and C250T. A fragment of 193 bp of the TERT promoter was amplified by PCR on about 100 ng of genomic DNA using primers 5'-CACCCGTCCTGCCCCTTCACCTT-3' (sense) and 5'-GGCTTCCCACGTGCGCAG CAGGA-3' (antisense). The thermal cycling conditions were as follows: initial denaturation for 3 min at 95 ° C, 40 cycles of denaturation at 95 ° C for 30 s, annealing at 68 ° C for 30 s, elongation at 72 ° C for 30 s and final primer extension at 72 ° C for 5 min. After quality confirmation by agarose gel electrophoresis, the PCR products were subjected to sanger sequencing performed using an ABI Prism 3730 DNA Analyzer (Applied Biosystems, Villebon sur Yvette, France).

BRAF V600E mutation analysis

Exon 15 of the BRAF gene containing the site for the T1799A (V600E) mutation was PCR amplified using primers 5'-TGCTTGCTCTGATAGGAAAATG-3' (sense) and 5'-AGCCTCAATTCTTACCATCCA-3' (antisense). PCR protocol comprised an initial denaturation of 3 min at 95 ° C, 40 amplification cycles (denaturation for 30 s at 95 ° C, annealing for 30 s at 60° C and extension for 30 s at 72° C) and final extension for 10 min at 72° C. After quality confirmation by agarose gel electrophoresis, the PCR products were subjected to Sanger sequencing analysis as described above for TERT mutations.

Statistical analysis

Statistical analyses were performed by R Statistical Software Package (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org). Continuous data are expressed as mean±standard deviation (SD) and median. Fisher's exact tests or chi-square tests

were used to test for the significance of categorical data, and unpaired *t* tests were used for continuous data. *P* value＜0.05 was considered to be statistically significant.

RESULTS

Clinical and molecular characteristics

The clinical and molecular characteristics of 66 DM-DTC patients included in this study are summarized in Table 1. Primary thyroid cancer samples include 64 papillary (PTC) and 2 follicular cases. The ratio of female to male was 1.36:1, relatively lower than sex ratio of DTC reported previously (*11*). Totally, only pulmonary metastases were involved in 59 patients, while 7 patients also had distant metastases to other organs.

Prevalence of TERT mutation and BRAF mutation in DM-DTC

The overall prevalence of TERT promoter mutations was 22.73% (15/66), of which C228T mutation was more prevalent (13/15, 86.67%) than C250T mutation (2/15, 13.33%). The coexistence of TERT and BRAF mutation was found in 10 patients (10/66, 15.15%), and other 5 patients (7.58%) were TERT mutation alone. BRAF V600E mutation was found in 29 cases (29/66, 43.94%) (Fig.1; Tables 1 and 2).

Association between TERT mutation and clinicopathological characteristics

TERT mutation was associated with older mean age at diagnosis (t=5.480, p<0.001), larger mean tumor diameter (t=2.803, p=0.013), and more likelihood of both BRAF mutation coexistence (χ^2=4.071, p=0.044) and refractory to RAI (χ^2=15.632, p＜0.001) (Table 1).

Association between TERT mutation and RAI imaging characteristics

A significant association of TERT mutation with RAI uptake pattern was observed (Table

3). TERT mutation associated with non-RAI-avid status, with a much lower T/B level than those with TERT wild-type obtained both from post radioiodine therapy whole-body scanning after initial RAI therapy (2.04 vs 18.54, $p<0.001$) and post radioiodine therapy whole-body scanning from subsequent RAI therapy when successful thyroid ablation was achieved (1.80 vs 12.30, $p<0.001$) (Table 3). Similarly, regardless of TERT mutation, BRAF mutation also associated with the non-RAI-avid status ($p<0.05$). Specifically, when TERT coexisted with BRAF mutation, the RAI uptake in metastatic foci was markedly reduced to near background level, with T/B levels significantly lower than only BRAF mutation group both at the first time (1.87 vs 9.1, $p=0.049$) and when there was no remnant thyroid (1.61 vs 7.43, $p=0.038$), which indicated a complete de-differentiation status (Table 3).

Association between TERT mutation and response to RAI therapy

Figure 2 showed that serological response differed significantly in 4 mutational groups (BRAF+TERT+, BRAF-TERT+, BRAF+TERT-, and BRAF-TERT-; Fisher's exact test，$p<0.05$). A rising sTg was noticed in 93.33% (14/15) of TERT mutation group, regardless of BRAF mutation. None of all 15 TERT mutation patients presented sTg decrease tendency. And BRAF mutation alone accounts for 52.63% of patients with sTg increase. Conversely, in cases with both mutations negative, 78.12% presented with decreased sTg and only 12.50% had sTg increased.

According to the criteria of RAI refractory, 33 RAI refractory cases were identified, and the other 33 cases were defined as disease-control group. TERT mutation closely correlated with RAI refractory, with a high specificity of 100% and a positive predictive value of 100%,

suggesting a powerful impact on an even worse clinical outcome (Fig. 1A). In addition, RAI responses of 4 different mutational scenarios are shown in Figure 1B.

Comparison of T/B level between BRAF mutation only and TERT mutation group

A total of 36 patients with detailed clinical information in our center were used for further T/B semi-quantitative evaluation (Fig. 3; Table 3). Patients with TERT mutation were compared with only BRAF mutation and no mutation in terms of T/B levels. TERT mutation group exhibited the lowest T/B level, only BRAF mutation group showed relatively lower level, comparing with both mutations negative group. Interestingly, 8 patients with TERT mutation all manifested as RAI refractory status even from the first time of RAI therapy, further confirmed by subsequent RAI therapy when successful remnant ablation were achieved. For 11 patients with only BRAF mutation, only 5 of them (45.45%) exhibited non-RAI-avidity initially, 2 (18.18%) of them gradually lost the ability of RAI trapping, thus eventually 7 of 11 (63.64%) were identified as RAI refractory status at the end of the follow-up time. While for 17 patients with both mutations negative, only 1 (5.88%) of them were non-RAI-avid initially and quickly refractory to RAI treatment, and most (94.12%, 16/17) of them presented with the function of RAI accumulation and manifested good response to RAI treatment.

Cases with different TERT/BRAF mutation status and RAI therapy response

Based upon the aforementioned scenarios, three representative cases were displayed in Figure 4, which showed distinct correlations between different TERT/BRAF mutation status and RAI uptake, as well as that between TERT/BRAF mutation status and RAI therapy response.

DISCUSSION

The prevalence of TERT promoter mutation was first reported in aggressive thyroid cancer by Xing et al. (*6*) in 2013. Soon afterwards, different studies demonstrated consistent association between TERT promoter mutation and poor clinicopathological characteristics such as older age, male, larger tumor size, extrathyroidal invasion, vascular invasion, distant metastasis, American Joint Committee on Cancer stage III/IV, recurrence, and mortality of follicular cell-derived thyroid cancer (*12-16*). The poor prognosis of DM-DTC is closely related to non-RAI-avidity in distant metastatic lesions and eventually induces RAI refractory status, thus poses a particularly tough prognostic and therapeutic challenge. Hence, early identification of lesions refractory to RAI could avoid repeated and time-consuming invalid RAI therapy and earn more time for alternative modalities. Molecular-based identification has been researched in studies, including BRAF mutation. In our previous study, BRAF mutation was demonstrated as a predictive molecular marker for non-RAI-avid status in DM-DTC, thus outlined a novel molecular-based non-RAI-avid prediction. And the coexistence of TERT and BRAF mutation formed a genetic background that defined PTC with the worst clinical outcomes (*14*), yet it remains unclear that how much TERT mutation may contribute to predict non-RAI-avidity and poor response to RAI therapy.

In this study, we found that the mean T/B level of RAI uptake in TERT mutation group was significantly lower than wild-type group. This result shows that TERT mutation holds a novel negative influence on RAI uptake status of distant metastatic lesions. Association between TERT promoter mutation and BRAF mutation has been reported in several studies (*13,14,17-19*) and it has been indicated that the coexistence of the two mutation types may

trigger more aggressive clinicopathological characteristics. BRAF mutation had been demonstrated as a predictor for non-RAI-avidity. However, differential imaging patterns could be observed when comparing between BRAF mutation alone and TERT mutation, with a 100% non-RAI-avid presence in TERT mutation group, indicating mutant TERT promoter might hold more potent negative influence on RAI uptake. T/B levels also confirmed that the coexistence of BRAF and TERT mutation may lead to the worst RAI uptake status, though larger sample size is still needed for further confirmation. Thus, for the first time, to our knowledge, we demonstrate that TERT mutation associates with both molecular-oriented radioiodine imaging pattern and non-RAI-avid status.

Several studies have demonstrated that non-RAI-avidity was distinctly associated with clinical outcome of DM-DTC (*3,20*). For instance, Song HJ et al. (*20*) reported that among patients with RAI-avid pulmonary metastases, 60.9% of them had significant decreased thyroglobulin levels after RAI therapy, and 60.3% of them had a reduction in metastases on follow-up computerized tomography. Whereas for non-RAI-avid patients, their survival rate significantly declined (*3,20*). It has been studied that the clinical characteristics, including older age (＜40 years old), lung combination with other distant metastatic organs, and larger tumor size, were correlated with both RAI uptake and RAI therapy response (*20*). While in this pioneering study, we suggested a novel molecular-based identification for both RAI uptake status and RAI therapy response of distant metastatic lesions. Our results manifested TERT promoter mutation could effectively predict the refractory status to RAI therapy with a positive predictive value as high as 100%, implying it associated with both RAI uptake characteristics

and clinical outcome. It might be used as an early indicator for non-RAI-avid status and poor response to RAI therapy, thus affords basis for tailoring the further management in these patients including timely stopping RAI therapy and offering more chance for other effective modalities, such as targeted therapy.

As thyroglobulin plays an important role on response evaluation, we further evaluated the response of TERT mutation in terms of sTg through a series of biochemical sTg surveillance. It was found that none of these 15 patients with TERT mutation presented sTg decrease tendency. By combining changes of sTg with RAI uptake status and other imaging evidence during follow-up, TERT mutant patients were all identified as refractory to RAI therapy at the end point of the follow-up. At present, it is still challenging for clinicians to judge the RAI refractory status early enough to tailor subsequent individual therapeutic schedule. We thus first presented this striking relationship between TERT mutation and RAI therapy response, which might shed light on molecular-driven individual management in advanced thyroid cancer.

There were studies showing that the prevalence of TERT promoter mutation exhibited significant variability among different countries ranging from 4.1% to 25.5% in PTC (*21-24*), and 5.9% to 36.4% in follicular thyroid cancer (*13,23*). Among these data, the Chinese prevalence was reported to be 4.1% to 11.3% in PTC (*13,21,24*), relatively lower than western countries. Whereas, this observation remains limited since there was only three studies conducted in China and no mutational data was documented in terms of DM-DTC, their RAI concentrative characteristics, and RAI therapy response. In present study, we first reported TERT mutation prevalence of 22.73% (15/66) in Chinese DM-DTC patients, of which C250T

mutation was also less frequent (13.33%, 2/15), consistent with previous studies. In contrast, TERT mutation incidence in DM-DTC patients is slightly more common in South Korea (40%, 12/30) and Italy (33%, 14/43) (*15,25*). Besides RAI refractory status, our data also showed that TERT mutation in DM-DTC patients associated with larger tumor size, older age, and more likelihood of BRAF mutation coexistence, in agreement with previously published data (*15*). These factors are also involved in aggressive characteristics of DM-DTC, reminding us that apart from molecular properties, multifactor-related mechanism might contribute to the progress of advanced thyroid cancer, especially for RAI refractory DTC.

Some limitations do exist in this retrospective study. First, the study cohort was relatively small, due to the low incidence of distant metastasis in DTC. Second, the low prevalence of TERT promoter mutation caused even smaller sample size in subgroups, thus prevented us from further persuasive statistics analysis.

CONCLUSIONS

TERT promoter mutation associates with non-RAI-avidity in DM-DTC, and when comparing with BRAF mutation, TERT mutation manifested a worse negative influence on RAI uptake. And it could be used as a novel molecular-based RAI refractory status predictor.

DISCLOSURE

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Figure legends

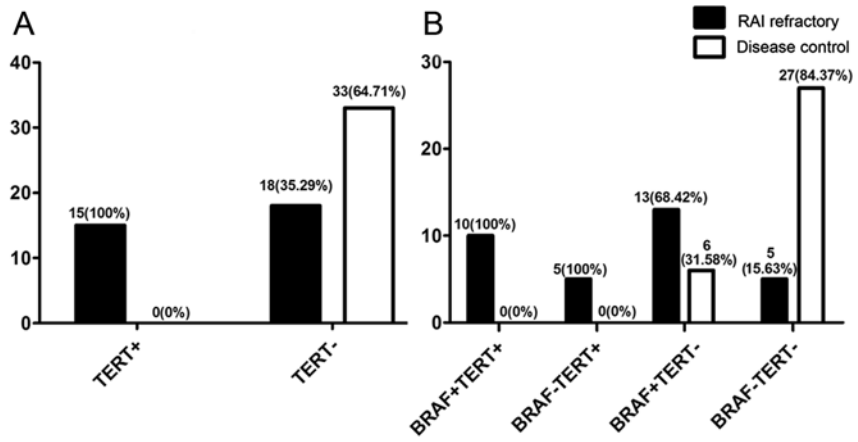

FIGURE 1. Association between TERT/BRAF mutation and RAI therapy response in DM-DTC. A. Association between TERT promoter mutation and RAI therapy response (RAI refractory or disease control). B. RAI responses of 4 different mutational scenarios. Note: "+" means mutation, "-" means wild-type.

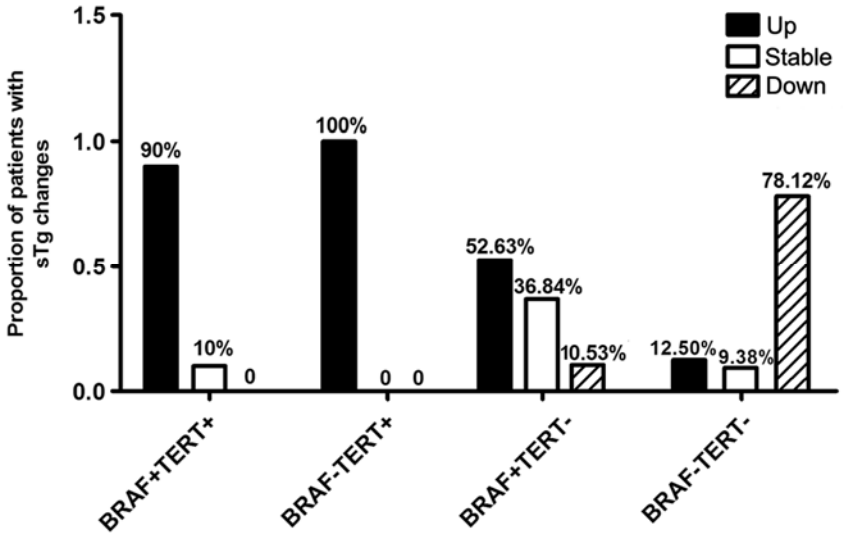

FIGURE 2. Serologic response to RAI therapy (up, stable, or down) of different mutational scenarios. Note: "+" means mutation, "-" means wild-type.

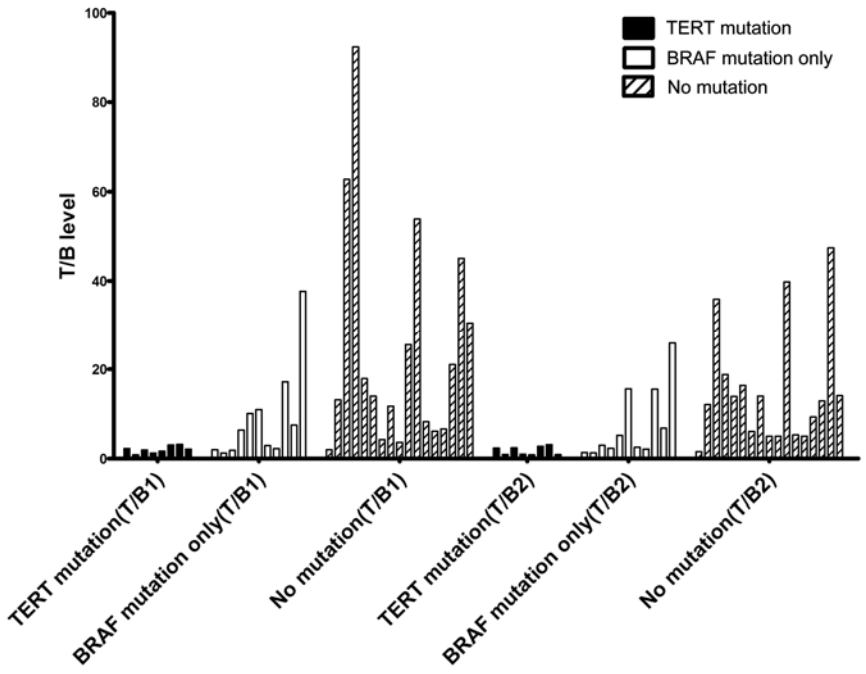

FIGURE 3. T/B levels of 36 DM-DTC cases. Note: T/B1, T/B level measured after the first RAI therapy with thyroid residue; T/B2, T/B level measured after subsequent RAI therapy without thyroid tissue remnant.

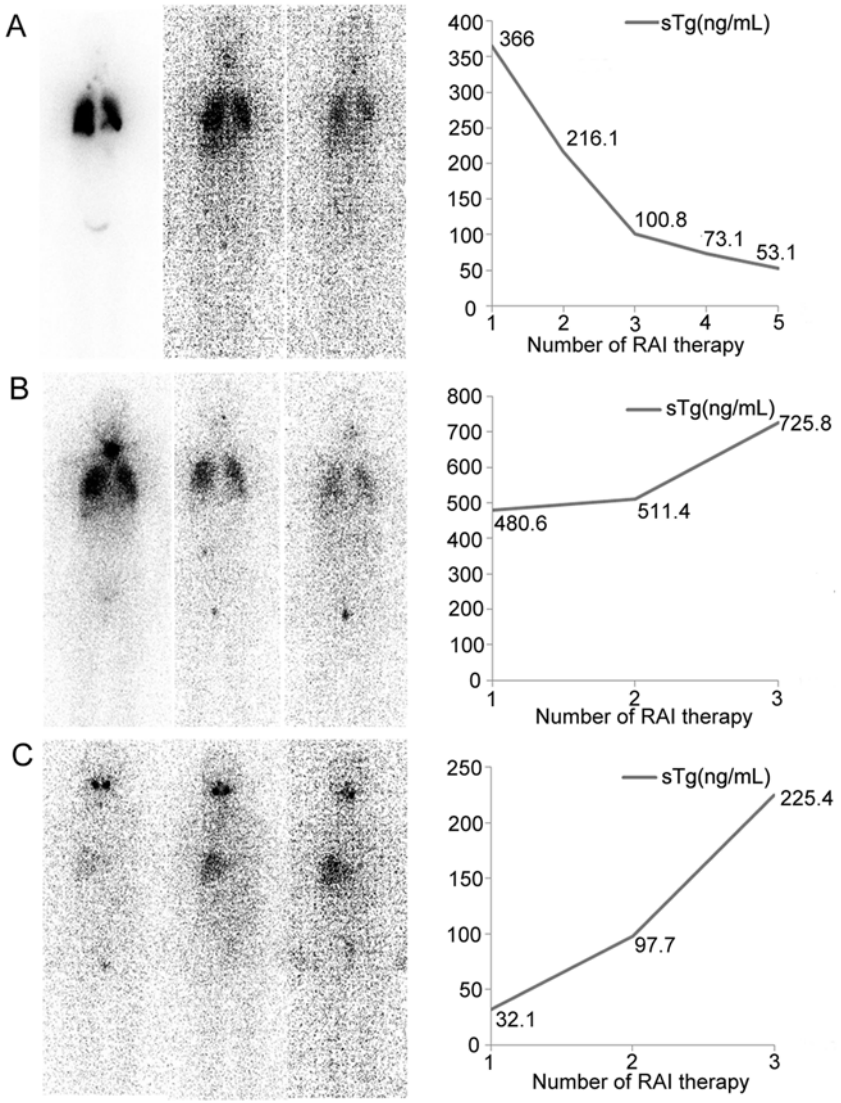

FIGURE 4. Three representative cases with different TERT/BRAF mutation status, RAI imaging and serologic responses. **A.** A 53-year old woman with pulmonary metastasis and both mutation negative. After RAI therapies, along with the gradually decreased RAI uptake over pulmonary metastatic foci, simultaneous declined sTg level was observed, indicating the efficacy of RAI therapy. **B.** A 34-year old woman with pulmonary metastasis and only BRAF mutation. STg level increased gradually, albeit along with less accumulation of RAI over metastatic foci, implying the trends of refractory to RAI. **C.** A 56-year old woman with pulmonary metastasis and harboring both TERT and BRAF mutation. The lung metastatic foci could not ever accumulate RAI and presented with sharply increased sTg level, indicating earlier inefficiency to RAI.

TABLE 1. Clinicopathological characteristics of 66 DTC patients with distant metastasis

Characteristic	$TERT^{MUT}$	$TERT^{WT}$	*p*
Number(n=66)	15	51	
Mean age ± SD (y)	58.27±10.51	36.86±13.98	**<0.001**
Sex			
Female	11	27	0.160
Male	4	24	
Mean tumor diameter ± SD(cm)	4.12±2.38	2.35±1.06	**0.013**
Histological diagnosis			
CPTC	6	18	
FV-PTC	4	24	
SD-PTC	3	6	
TCV-PTC	1	1	
HV-PTC	0	1	
FTC	1	1	
Aggressive histologic subtypes	5	9	0.344
Less aggressive histologic subtypes *	10	42	
Multifocality			
One lesion	3	15	0.697
More than one lesion	12	36	
Extrathyroidal invasion			
Yes	13	39	0.624
No	2	12	
Lymph node metastasis			
N0	0	2	
N1a	0	0	
N1b	12	44	
Nx	3	5	
Site of distant metastases			
Lung	12	47	0.386
Lung + other organs	3	4	
BRAFV600E mutation			
Yes	10	19	**0.044**
No	5	32	
Refractory to RAI			
Yes	15	18	**<0.001**
No	0	33	

Abbreviations: MUT: mutation; WT: wild-type; SD: standard deviation; CPTC: classic PTC; FV-PTC: follicular-variant PTC; SD-PTC: sclerosing diffuse PTC; TCV-PTC: tall cell-variant PTC; HV-PTC: hobnail-variant PTC; FTC: follicular thyroid cancer; Nx: Patients didn't experience cervical lymph nodes dissection. Note: * CPTC and FV-PTC were considered as less aggressive histologic subtypes, and the

others were considered as aggressive histologic subtypes.

TABLE 2. Association between BRAFV600E alone or TERT promoter mutation alone or their coexistence and clinicopathological characteristics of DTC

	No mutation	TERT mutation Only	BRAF mutation Only	TERT+BRAF mutation
Total DTC(n=66)	32(48.48%)	5(7.58%)	19(28.79%)	10(15.15%)
Age± SD (y)	35.00±14.73	61.80±8.43	40.00±12.34	56.50±11.39
Sex				
Females	15	4	12	7
Males	17	1	7	3
Histological diagnosis				
CPTC	11	2	7	4
FV-PTC	16	1	8	3
SD-PTC	4	1	2	2
TCV-PTC	0	0	1	1
HV-PTC	0	0	1	0
FTC	1	1	0	0
TNM stage				
II	23	0	11	2
IV	9	5	8	8
Refractory to RAI				
Yes	5	5	13	10
No	27	0	6	0

TABLE 3. T/B level measured after the first RAI therapy and successful thyroid ablation in different mutation groups

	T/B1	*p*	T/B2	*p*
Irrespective of BRAF				
TERT+	2.04±0.82	**＜0.001**	1.80±0.97	**＜0.001**
TERT-	18.54±21.84		12.30±12.01	
Irrespective of TERT				
BRAF+	6.29±8.97	**0.012**	5.17±6.86	**0.011**
BRAF-	23.45±24.92		14.76±13.19	
BRAF+				
BRAF+TERT+	1.87±0.72	**0.049**	1.61±0.87	**0.038**
BRAF+TERT-	9.1±10.69		7.43±8.07	
BRAF-				
BRAF-TERT+	3.22*	0.420	3.13	0.380
BRAF-TERT-	24.64±25.15		15.44±13.26	

Abbreviations: T/B: tumor/background ratio. T/B1: T/B level measured after the first RAI therapy with thyroid residue; T/B2: T/B level measured after subsequent RAI therapy without thyroid tissue remnant.
Note: *only one patient with TERT mutation alone was available with T/B level.