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LE THI TUYET NHUNG

**CLINICAL CHARACTERISTICS,
HISTOPATHOLOGICAL FEATURES, *BRAF* V600E,
TERT PROMOTER MUTATIONS IN PATIENTS WITH
RADIOIODINE-REFRACTORY DIFFERENTIATED
THYROID CARCINOMA**

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1. Le Thi Tuyet Nhung, Le Ngoc Ha, Hoang Van Tong, et al. (2025). Prognostic Significance of *BRAF* V600E and *TERT* Promoter Mutations in Radioiodine Resistance and Recurrence of Differentiated Thyroid Cancer. *Medicine*, 104(38): e44540.

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INTRODUCTION

Thyroid cancer (TC) is the most common malignant disease of the endocrine system and ranks twelfth among cancers worldwide. Patients with differentiated thyroid carcinoma (DTC) generally have a 10-year survival rate exceeding 90% after treatment. However, approximately 10-30% of TC patients may progress with recurrence or distant metastasis, accompanied by decreased expression of the sodium iodide symporter (NIS), reduced targeting of NIS to the cell membrane due to dysfunction, or even loss of NIS expression at the basolateral membrane. This phenomenon, characterized by loss of radioiodine (RAI) avidity, is defined as radioiodine-refractory differentiated thyroid carcinoma (RAIR-DTC). This issue has attracted significant interest in research and clinical practice because the 10-year survival rate of RAIR-DTC patients is below 10%, and the 5-year survival rate is less than 50%. Genetic alterations play an important role in tumorigenesis and thyroid cancer pathogenesis through aberrant activation of protein kinase pathways, including the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways. These alterations are associated with suppression of several iodide-handling genes, particularly SLC5A5 encoding NIS, leading to therapeutic failure with RAI. Advanced RAIR-TC with metastasis generally carries a poor prognosis. Therefore, molecular biomarkers such as BRAFV600E mutation and telomerase reverse transcriptase (TERT) promoter mutation are of major importance in diagnosis, treatment, and follow-up of DTC. Notably, recent studies have reported that concurrent

BRAFV600E and TERT promoter mutations are strongly associated with RAI refractoriness, reduced iodide metabolism, and aggressive disease in recurrent papillary thyroid carcinoma, thereby contributing to unfavorable prognosis in RAI-RDTC. Assessing the role of BRAFV600E and TERT promoter mutations, including their co-occurrence and association with aggressive variants, supports better diagnostic accuracy, prognostic evaluation, and development of appropriate therapeutic strategies for RAI-RDTC patients in clinical practice.

In Vietnam, studies on genetic mutations in RAI-RDTC remain limited, with few clinical applications. Therefore, we conducted this study with the following objectives:

- 1. To evaluate clinical features, histopathological characteristics, and the frequency of BRAFV600E and TERT promoter mutations in patients with radioiodine-refractory differentiated thyroid carcinoma.*
- 2. To analyze the correlation between clinical features, histopathological findings, treatment outcomes, and mutation/co-mutation status of BRAFV600E and TERT promoter in patients with RAI-RDTC.*

CONTRIBUTIONS OF THE THESIS

- The findings of this study add further evidence on the clinical and histopathological characteristics, as well as the frequency of *BRAF* V600E and *TERT* promoter mutations, thereby contributing to the clinical practice of diagnosis, treatment, and prognosis of recurrent radioiodine-refractory differentiated thyroid carcinoma.

Male sex, age > 55 years, T4 tumor size, and elevated Tg levels were identified as clinical factors associated with an increased risk of disease progression.

- The study provides fundamental insights into the outcomes of surgery for recurrent disease, particularly highlighting that the co-occurrence of *BRAF/TERT* mutations is an independent prognostic factor influencing radioiodine refractoriness and post-reoperation disease progression in patients with recurrent differentiated thyroid carcinoma in the neck with radioiodine resistance.

THESIS STRUCTURE

The dissertation comprises 127 pages (excluding appendices and references) and is organized into four main chapters: Introduction (3 pages), chapter 1 - Literature Review (34 pages), chapter 2 - Subjects and Methods (22 pages), chapter 3 - Results (35 pages), and chapter 4 - Discussion (30 pages), followed by Conclusion and Recommendations (3 pages). The dissertation includes 27 tables, 11 charts, 6 illustrative figures, 1 diagram, and 120 references (4 in Vietnamese and 116 in English).

CHAPTER 1. LITERATURE REVIEW

1.1. General characteristics of radioiodine-refractory differentiated thyroid carcinoma (RAIR-DTC)

1.1.1. Definition of RAIR-DTC

According to the 2015 American Thyroid Association (ATA) guidelines, RAIR-DTC is defined by four categories: Type 1: Malignant lesions or metastases do not concentrate radioiodine

(RAI); whole-body scan after initial RAI therapy shows no uptake at metastatic sites outside the thyroid bed. Type 2: Lesions lose the ability to concentrate RAI after previously demonstrating uptake (excluding patients with long-term iodine exposure). Type 3: Mixed uptake, where some lesions concentrate RAI while others do not, often observed in patients with bulky, multiple metastases. Type 4: Progressive disease despite substantial RAI uptake (new lesions, enlargement of existing lesions, or elevated serum thyroglobulin within a few months after RAI therapy).

1.1.2. Risk stratification for recurrence

According to ATA 2015, recurrence risk is stratified into low, intermediate, and high categories based on clinical and pathological features.

1.1.3. Clinical manifestations of RAIR-DTC

Advanced thyroid carcinoma invading adjacent structures is a major cause of recurrence and mortality. Cervical lymph node metastases may infiltrate large veins, bones, sternocleidomastoid muscle, pleura, lungs, and rarely the carotid artery. Clinical symptoms include hoarseness, dyspnea, severe neck pain, dysphagia, Horner's syndrome, and skin invasion.

1.1.4. Ancillary investigations in RAIR-DTC

1.1.4.1. Serum thyroglobulin

1.1.4.2. Neck ultrasound

1.1.4.3. Fine-needle aspiration

1.1.4.4. Whole-body RAI scan

1.1.4.5. CT and MRI

1.1.4.6. 18F-FDG PET/CT

1.1.4.7. Histopathology

The 2017 World Health Organization (WHO) classification of endocrine tumors introduced several updates compared with previous editions. Tall cell variant papillary thyroid carcinoma is characterized by the predominance (>50%) of tumor cells whose height is at least three times their width. Columnar cell variant papillary thyroid carcinoma is defined by the predominance of columnar cells with prominent nuclear stratification. Hobnail variant papillary thyroid carcinoma, a rare subtype, is characterized by the predominance of hobnail-shaped cells with apically located nuclei and bulging apical surfaces. Other variants of papillary thyroid carcinoma with poorer prognosis include the solid variant and the diffuse sclerosing variant.

1.1.5. Staging at initial diagnosis

Application of the 8th edition AJCC staging system for the assessment of primary tumor (T: Tumor), lymph node status (N: Lymph Node), and distant metastasis (M: Metastasis).

In addition to these key parameters for patient staging (T, N, M), the 8th edition also introduces supplementary clinical factors that are considered to further support risk stratification.

1.1.6. Treatment of RAIR-DTC

1.1.6.1. Suppressive thyroid hormone therapy with TSH suppression.

1.1.6.2. Local therapy: Surgery, external beam radiotherapy, percutaneous techniques (radiofrequency ablation, laser ablation, ethanol injection, cementoplasty for bone metastases).

1.1.6.3. Systemic therapy: RAI therapy (if partially responsive), targeted therapy (tyrosine kinase inhibitors), and immunotherapy.

1.2. Molecular mechanisms of RAIR-DTC

1.2.1. Role of the sodium iodide symporter (NIS)

Iodide uptake occurs across the thyroid follicular cell membrane via active transport mediated by the sodium/iodide symporter (Na^+/I^- symporter, NIS). The expression of NIS is inversely correlated with the degree of differentiation of thyroid cancer cells. Dedifferentiation of thyroid cancer cells is a key mechanism leading to disease progression, metastasis, and refractoriness to radioactive iodine (RAI) therapy.

1.2.2. Major signaling pathways

MAPK and PI3K/AKT pathways plays a central role

1.2.3. Other signaling pathways

1.3. Role of *BRAF* V600E and *TERT* promoter mutations in RAIR-DTC

1.3.1. BRAF V600E mutation

The *BRAF* V600E mutation has been shown to be associated with the loss of RAI avidity in recurrent papillary thyroid carcinoma (PTC), attributable to the mislocalization of the sodium/iodide symporter within the cytoplasm of thyroid cancer cells. *BRAF* V600E may interfere with RAI uptake at distant metastatic sites of PTC, and its presence in the primary tumor serves as a molecular marker for predicting RAI uptake in distant metastases.

1.3.2. TERT promoter mutation

TERT promoter mutations may co-occur with *BRAF* mutations and are associated with loss of radioiodine uptake in patients with metastatic differentiated thyroid carcinoma (DTC). Therefore, they may serve as an early predictive marker of radioiodine-refractory differentiated thyroid carcinoma (RAIR-DTC).

1.3.3. BRAFV600E/TERT co-mutation

The synergistic effect of *BRAF* V600E and *TERT* promoter mutations on thyroid cancer progression may be related to tumor molecular alterations that promote invasiveness within the thyroid cancer microenvironment.

1.4. Current research on BRAFV600E and TERT promoter mutations in RAIR-DTC

1.4.1. International studies

1.4.1.1. BRAF V600E mutation

A recent study in Korea by Kim et al. (2022) involving 7,797 patients with papillary thyroid carcinoma (PTC) reported that 84% harbored the *BRAF* V600E mutation, 1.1% had *TERT* promoter mutations, and 0.9% presented with concurrent *BRAF/TERT* promoter mutations. The *BRAF* V600E mutation was more frequently observed in males ($p < 0.001$), tumor size ≤ 1 cm ($p < 0.001$), conventional PTC ($p < 0.001$), node-negative disease ($p = 0.005$), stage N1 ($p = 0.009$), and M0 status ($p < 0.001$).

1.4.1.2. TERT promoter mutation

Cheng (2023) proposed a high-risk prediction model for patients with metastatic PTC, which included the following factors: primary tumor T3/T4, >5 metastatic regional lymph nodes, and co-

occurrence of *BRAF/TERT* promoter mutations. Kim (2022) demonstrated that *TERT* promoter mutations were significantly associated with age ≥ 55 years, tumor size > 1 cm, multifocal PTC, extrathyroidal extension, PTC variants, T3–T4 stage, M1 disease, stage III–IV, and adjuvant RAI therapy.

1.4.1.3. Co-mutations of BRAF V600E and TERT promoter

Liu J. et al. (2019) reported that the risk of loss of RAI avidity was significantly higher in tumors with concurrent *BRAF/TERT* promoter mutations compared with either mutation alone. Synergistic analysis revealed that the risk of radioiodine refractoriness increased 13.28-fold (95% CI, 1.54–114.46; $p = 0.019$) in the presence of both *BRAF* and *TERT* promoter mutations.

1.4.2. Domestic studies

Recent studies by Ngô Thị Minh Hạnh (2020), Nguyễn Thị Lan Hương (2023), and Đặng Trung Dũng (2023) demonstrated no association between *BRAF* V600E mutation status and the risk of recurrence, lymph node metastasis, distant metastasis, or disease progression in patients with radioiodine-refractory differentiated thyroid carcinoma (RAIR-DTC). To date, in Vietnam, research on *BRAF* V600E and *TERT* promoter mutations, as well as their co-occurrence in patients with recurrent, RAIR-DTC in the cervical region, remains limited and has not been extensively addressed in the literature.

Chapter 2. SUBJECTS AND STUDY METHODS

2.1. Study object

The study was conducted on 111 patients with recurrent, radioiodine-refractory differentiated thyroid carcinoma (RAIR-DTC) in the cervical region, compared with a reference group of 33 patients with recurrent, radioiodine-avid DTC in the cervical region.

2.1.1. Recurrent cervical radioiodine-refractory DTC group

The study group included 111 patients with RAIR-DTC who underwent surgery for recurrent cervical lesions at the 108 Military Central Hospital. Paraffin-embedded tissue samples from these recurrent lesions were obtained for histopathological and molecular analyses.

2.1.2. Recurrent cervical radioiodine-avid DTC group

The reference group consisted of 33 patients diagnosed with recurrent cervical DTC that demonstrated radioiodine uptake on post-treatment whole-body I-131 scintigraphy. These patients underwent surgical resection of recurrent cervical lesions with available histopathological and molecular samples.

2.1.3. Inclusion criteria

2.1.3.1. Study group

Patients were followed up and detected with recurrent cervical lesions, diagnosed as RAIR-DTC, and subsequently underwent surgical resection of the recurrent cervical lesions. Postoperative histopathology confirmed metastatic thyroid carcinoma. Complete medical records, slides, and paraffin tissue blocks were available.

2.1.3.2. Reference group: Patients with recurrent cervical lesions that demonstrated I-131 uptake on whole-body scintigraphy. Complete medical records, slides, and paraffin blocks were available and eligible for genetic mutation testing.

2.1.4. Exclusion criteria: Patients with a primary tumor histology other than differentiated thyroid carcinoma. Patients with concomitant malignancies.

2.2. Study location

Departments of Nuclear Medicine, Thoracic Surgery, Pathology, and Molecular Biology, 108 Military Central Hospital.

2.3. Study period: From January 2021 to December 2024.

2.4. Research methods

2.4.1. Study design

A cross-sectional descriptive study combining retrospective and prospective analyses with a 12-month longitudinal follow-up after neck dissection.

2.4.2. Sample size: The minimum required sample size for the RAIR-DTC group in this study was 95 patients.

2.4.3. Research facilities

Laboratory equipment at the Departments of Clinical Laboratory, Diagnostic Imaging, Nuclear Medicine, and Molecular Biology, 108 Military Central Hospital.

2.4.4. Study variables

2.4.5. Procedures and techniques for genetic mutation analysis

2.5. Data management and statistical analysis

2.6. Ethical considerations

Chapter 3. STUDY RESULTS

3.1. Clinical, histopathological characteristics, and prevalence of BRAF V600E and TERT promoter mutations in patients with radioiodine-refractory differentiated thyroid carcinoma (RAIR-DTC)

3.1.4. Association between clinical features, histopathology, gene mutations, and I-131 uptake status in recurrent cervical lesions

Table 3.11. Association between BRAF V600E, TERT promoter mutations, and I-131 uptake status in recurrent cervical lesions

Gene mutation		Total group (n=144)	RAIR-DTC (n=111)	Radioiodine-avid DTC (n=33)	OR (95% CI)	P
BRAF	(-)	26 (18.1)	18 (16.2)	8 (24.2)	-	Ref
	(+)	118 (81.9)	93 (83.8)	25 (75.8)	1.7 (0.6 – 4.2)	0.3 ^a
TERT	(-)	89 (61.8)	62 (55.9)	27 (81.8)	-	Ref
	(+)	55 (38.2)	49 (44.1)	6 (18.2)	3.6 (1.3 – 11.3)	0.007^a
No mutation		20 (13.9)	12 (10.8)	8 (24.2)	-	Ref
Only BRAF+		69 (47.9)	50 (45.1)	19 (57.6)	1.8 (0.6 – 5.5)	0.29 ^a
Only TERT+		6 (4.2)	6 (5.4)	0 (0.0)	NA	NA
BRAF+/TERT+		49 (34.0)	43 (38.7)	6 (18.2)	4.8 (1.2 – 19.9)	0.009^a

^aChi-square test; Ref: Reference; NA: Not applicable

Comment:

The frequency of *TERT* promoter mutations was significantly higher in RAIR-DTC compared with radioiodine-avid DTC. *BRAF/TERT* co-mutations were detected in 34% (49/144) of patients

with differentiated thyroid carcinoma. The co-mutation rate was significantly higher in RAIR-DTC than in radioiodine-avid DTC ($p = 0.009$).

3.2. Analysis of the association between clinical features, histopathology, treatment outcomes, and the status of BRAF V600E and TERT promoter mutations/co-mutations in patients with radioiodine-refractory differentiated thyroid carcinoma (RAIR-DTC)

3.2.1. Association between clinical features, histopathology, treatment outcomes, and the status of BRAF V600E and TERT promoter mutations/co-mutations

Table 3.14. Comparison of characteristics among groups with wild-type *BRAF*, *BRAF* mutation only, and *BRAF/TERT* co-mutation

Characteristics		Gene mutation			P
		<i>BRAF</i> (-) (n=18), n (%)	<i>BRAF</i> (+) (n=50), n (%)	<i>BRAF/TERT</i> (n=43), n (%)	
Age at diagnosis	Mean $\pm$ SD	44.2 $\pm$ 11.2	40.6 $\pm$ 13.3	50.1 $\pm$ 13.8	0,003^a
	< 55	14 (77.8)	41 (82.0)	26 (60.5)	0,06 ^c
	$\geq$ 55	4 (22.2)	9 (18.0)	17 (39.5)	
Age at recurrence	TB $\pm$ SD	49.6 $\pm$ 9.1	45.2 $\pm$ 13.2	56.4 $\pm$ 13.8	0,00^a
	< 55	12 (66.7)	36 (72.0)	18 (41.9)	0,01^c
	$\geq$ 55	6 (33.3)	14 (28.0)	25 (58.1)	
Sex	Female	11 (61.1)	41 (82.0)	31 (72.1)	0,19 ^c
	Male	7 (38.9)	9 (18.0)	12 (27.9)	
Tumor T4	Yes	1 (5.6)	1 (2.0)	12 (27.9)	0,001^c
	No	17 (94.4)	49 (98.0)	31 (72.1)	
Lymph node metastasis	N0	4 (22.2)	11 (22.0)	9 (20.9)	1,0 ^d
	N1	14 (77.8)	38 (76.0)	34 (79.1)	
	Nx	0 (0.0)	1 (2.0)	0 (0.0)	
Distant metastasis	M0	18 (100.0)	50 (100.0)	43 (100.0)	-
	M1	0 (0.0)	0 (0.0)	0 (0.0)	
Risk of recurrence	Low	1 (5.6)	2 (4.0)	0 (0.0)	0,02^d
	Intermediate	8 (44.4)	15 (30.0)	6 (14.0)	
	High	9 (50.0)	33 (66.0)	37 (86.0)	
I-131 dose (mCi)	Median (IQR)	252.5 (218.8 – 318.8)	275 (195 – 350)	300 (250 – 450)	0,14 ^b
	< 600	16 (88.9)	47 (94.0)	38 (88.4)	0,60 ^d
	$\geq$ 600	2 (11.1)	3 (6.0)	5 (11.6)	
Number of I-131 treatments	< 3	14 (77.8)	35 (70.0)	24 (55.8)	0,08 ^d
	3 – 5	3 (16.7)	13 (26.0)	19 (44.2)	
	$\geq$ 6	1 (5.6)	2 (4.0)	0 (0.0)	

SuppressedTg (ng/ml)	Median (IQR)	0.9 (0.1 – 1.9)	1.2 (0.1 – 3.2)	4.3 (0.5 – 16.8)	0,005^b
	< 1	9 (50.0)	23 (46.9)	13 (30.2)	0,19 ^c
	≥ 1	9 (50.0)	26 (53.1)	30 (69.8)	
Stimulated Tg (ng/ml)	Median (IQR)	7.9 (0.4 – 36.0)	27.8 (4.8 – 74.1)	38.2 (14.4 – 131.5)	0,02^b
	< 10	10 (55.6)	15 (31.3)	8 (19.5)	0,02^c
	≥ 10	8 (44.4)	33 (68.8)	33 (80.5)	
Histopathology of recurrent tumor	Convention al papillary	15 (83.3)	38 (76.0)	37 (86.0)	0,45 ^c
	Other variants	3 (16.7)	12 (24.0)	6 (14.0)	

^a one-way ANOVA test; ^b Kruskal Wallis test; ^c Chi-square test; ^d Fisher's exact test

Comment:

There were statistically significant associations between the *BRAF*-negative, *BRAF*-positive, and *BRAF/TERT* co-mutation groups and the following variables: patient age at diagnosis, age at recurrence, T4 tumor size, risk of recurrence, pre I-131 stimulated Tg, and suppressed Tg at the time of recurrence ($p < 0.05$).

Table 3.15. Comparison of characteristics among groups with wild-type *TERT*, *TERT* mutation only, and *BRAF/TERT* co-mutation

Characteristics		Gene mutation			p
		<i>TERT</i> (–) (n=62, n (%))	<i>TERT</i> (+) (n=6, n (%))	<i>BRAF/TERT</i> (n=43, n (%))	
Age at diagnosis	Mean ± SD	41.2 ± 13.1	44.7 ± 9.7	50.1 ± 13.8	0,005^a
	< 55	50 (80.6)	5 (83.3)	26 (60.5)	0,054 ^d
	≥ 55	12 (19.4)	1 (16.7)	17 (39.5)	
Age at recurrence	Mean ± SD	46.0 ± 12.7	49.5 ± 8.7	56.4 ± 13.8	0,001^a
	< 55	44 (71.0)	4 (66.7)	18 (41.9)	0,01^d
	≥ 55	18 (29.0)	2 (33.3)	25 (58.1)	
Sex	Female	50 (80.6)	2 (33.3)	31 (72.1)	0,03^d
	Male	12 (19.4)	4 (66.7)	12 (27.9)	
Tumor T4	Yes	2 (3.2)	0 (0.0)	12 (27.9)	0,001^c
	No	60 (96.8)	6 (100.0)	31 (72.1)	
Lymph node metastasis	N0	14 (22.6)	1 (16.7)	9 (20.9)	1,0 ^d
	N1	47 (75.8)	5 (83.3)	34 (79.1)	
	Nx	1 (1.6)	0 (0.0)	0 (0.0)	
Distant metastasis	M0	62 (100.0)	6 (100.0)	43 (100.0)	-
	M1	0 (0.0)	0 (0.0)	0 (0.0)	
Risk of recurrence	Low	2 (3.2)	1 (16.7)	0 (0.0)	0,007^d
	Intermediate	20 (32.3)	3 (50.0)	6 (14.0)	
	High	40 (64.5)	2 (33.3)	37 (86.0)	
I-131 dose (mCi)	Median (IQR)	275 (218.8 - 350)	225 (181.3 – 368.8)	300 (250 - 450)	0,09 ^b
	< 600	58 (93.5)	5 (83.3)	38 (88.4)	0,37 ^d
	≥ 600	4 (6.5)	1 (16.7)	5 (11.6)	
	< 3	44 (71.0)	5 (83.3)	24 (55.8)	0,13 ^d
	3 – 5	15 (24.2)	1 (16.7)	19 (44.2)	

	≥ 6	3 (4.8)	0 (0.0)	0 (0.0)	
Suppressed Tg (ng/ml)	Median (IQR)	1.2 (0.1 – 3.2)	0.1 (0.1 – 1.5)	4.3 (0.5 – 16.8)	0,003 ^b
	< 1	28 (45.9)	4 (66.7)	13 (30.2)	
	≥ 1	33 (54.1)	2 (33.3)	30 (69.8)	0,14 ^d
Stimulated Tg (ng/ml)	Median (IQR)	26.0 (3.7 - 72.8)	5.0 (1.1 – 93.0)	38.2 (14.4 – 131.5)	0,10 ^b
	< 10	21 (35.0)	4 (66.7)	8 (19.5)	0,04 ^d
	≥ 10	39 (65.0)	2 (33.3)	33 (80.5)	
Histopathology of recurrent tumor	Conventional papillary	48 (77.4)	5 (83.3)	37 (86.0)	0,57 ^d
	Other variants	14 (22.6)	1 (16.7)	6 (14.0)	

^a one-way ANOVA test; ^b Kruskal Wallis test; ^c Chi-square test; ^d Fisher's exact test

Comment:

Statistically significant differences ($p < 0.05$) were observed among the *TERT*-negative, *TERT*-positive, and *BRAF/TERT* co-mutation groups in terms of patient age at diagnosis, age at recurrence, T4 tumor size, high risk of recurrence, pre I-131 stimulated Tg, and suppressed Tg at the time of recurrence.

Table 3.17. Comparison of histopathological characteristics of recurrent cervical lesions according to *BRAF/TERT* co-mutation status

Histopathological type		<i>BRAF/TERT</i> co-mutation (n=43), n (%)	No co-mutation (n=68), n (%)	p
Intermediate papillary variants	Conventional papillary	37 (86.0)	53 (77.9)	0.14 ^a
	Oncocytic variant	1 (2.3)	2 (2.9)	
	Follicular variant	1 (2.3)	8 (11.8)	
	Clear cell variant	0 (0.0)	3 (4.4)	
Aggressive papillary variants	Tall cell variant	2 (4.7)	1 (1.5)	
	Columnar cell variant	0 (0.0)	1 (1.5)	
	Hobnail variant	1 (2.3)	0 (0.0)	
	Diffuse sclerosing variant	1 (2.3)	0 (0.0)	

^a Fisher's exact test

Comment:

In the *BRAF/TERT* co-mutation group, the tall cell variant accounted for 4.7%, the hobnail variant 2.3%, and the diffuse sclerosing variant 2.3%, which were higher compared with the no co-mutation group (1.5% and 0.0%, respectively). However, these differences were not statistically significant ($p > 0.05$).

Table 3.18. Comparison of microscopic features of recurrent cervical lesions according to *BRAF/TERT* co-mutation status

Microscopic features		<i>BRAF/TERT</i> co-mutation (n=43), n (%)	No co-mutation (n=68), n (%)	P
Vascular invasion	Yes	0 (0.0)	4 (5.9)	0,16 ^a
	No	43 (100.0)	64 (94.1)	
Necrosis	Yes	7 (16.3)	6 (8.8)	0,23 ^b
	No	36 (83.7)	62 (91.2)	
Capsular invasion	Yes	0 (0.0)	1 (1.5)	1,0 ^a
	No	43 (100.0)	67 (98.5)	
Muscle invasion	Yes	4 (9.3)	1 (1.5)	0,07 ^a
	No	39 (90.7)	67 (98.5)	
Perinodal soft tissue invasion	Yes	11 (25.6)	14 (20.6)	0,54 ^b
	No	32 (74.4)	54 (79.4)	
Calcification	Yes	4 (9.3)	11 (16.2)	0,30 ^b
	No	39 (90.7)	57 (83.8)	
Perineural invasion	Yes	1 (2.3)	0 (0.0)	0,39 ^a
	No	42 (97.7)	68 (100.0)	

^a Fisher's exact test, ^b Chi-square test

Comment:

Analysis of microscopic features in recurrent lymph node tissue of the *BRAF/TERT* co-mutation group showed necrosis in 16.3%, muscle invasion in 9.3%, perinodal soft tissue invasion in 25.6%, and perineural invasion in 2.3%. These rates were higher than in the no co-mutation group, which had 8.8%, 1.5%, 20.6%, and

0.0%, respectively. However, the differences were not statistically significant ($p > 0.05$).

Table 3.22. Cox regression analysis of clinical factors and gene mutations in relation to disease progression after reoperation for recurrent lesions

Factors	Univariate analysis			Multivariate analysis		
	<i>HR</i>	<i>95% CI</i>	<i>p</i>	<i>HR</i>	<i>95% CI</i>	<i>p</i>
Age at recurrence $\geq$ 55 years	3.0	1.2 – 7.6	0.018	1.7	0.6 – 4.7	0.308
Male sex	3.4	1.4 – 8.2	0.006	3.6	1.5 – 8.8	0.005
Distant recurrence	8.1	2.9 – 22.6	0.001	4.1	1.4 – 12.6	0.013
Suppressed Tg at recurrence $\geq$ 1 ng/mL	4.3	1.3 – 14.8	0.019	2.9	0.8 – 10.5	0.096
<i>BRAF/TERT</i> co-mutation	4.3	1.7 – 11.2	0.003	3.3	1.2 – 9.2	0.023

Comment:

Multivariate analysis revealed that only the clinical factors male sex ($HR = 3.6$; 95% CI : 1.5–8.8; $p = 0.005$), distant recurrence (lung or bone metastases) ($HR = 4.1$; 95% CI : 1.4–12.6; $p = 0.013$), and *BRAF/TERT* promoter co-mutation ($HR = 3.3$; 95% CI : 1.2–9.2; $p = 0.023$) were independent prognostic factors associated with an increased risk of disease progression after surgical resection of recurrent cervical lesions in patients with radioiodine-refractory differentiated thyroid carcinoma (RAIR-DTC).

3.2.2. Prognostic value of selected clinical features, histopathology, treatment outcomes, and the status of BRAF V600E and TERT promoter mutations/co-mutations in patients with radioiodine-refractory differentiated thyroid carcinoma

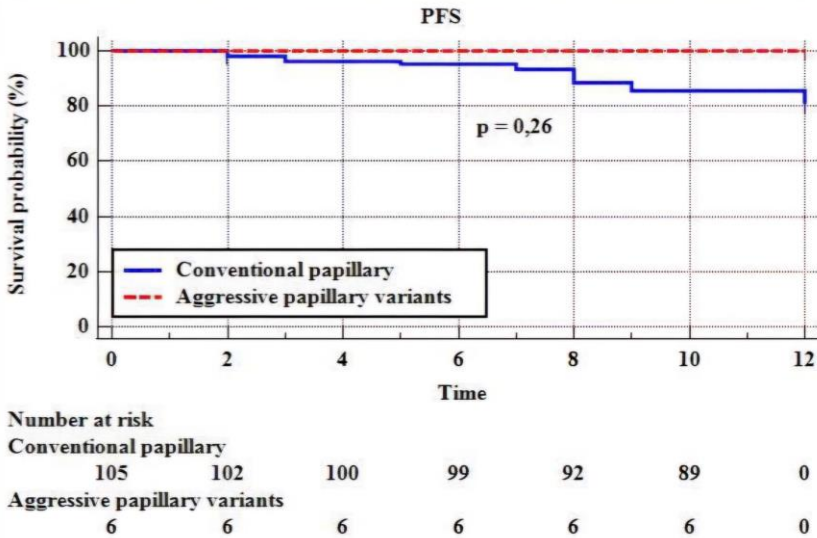


Figure 3.6. Kaplan–Meier curve comparing progression-free survival after surgery for recurrence according to histopathological subtype

Comment:

In radioiodine-refractory differentiated thyroid carcinoma (RAI-R DTC), the recurrence of conventional papillary carcinoma showed no statistically significant difference in progression-free survival (PFS) after reoperation compared with the progressive variant of papillary carcinoma, with $p = 0.26 > 0.05$.

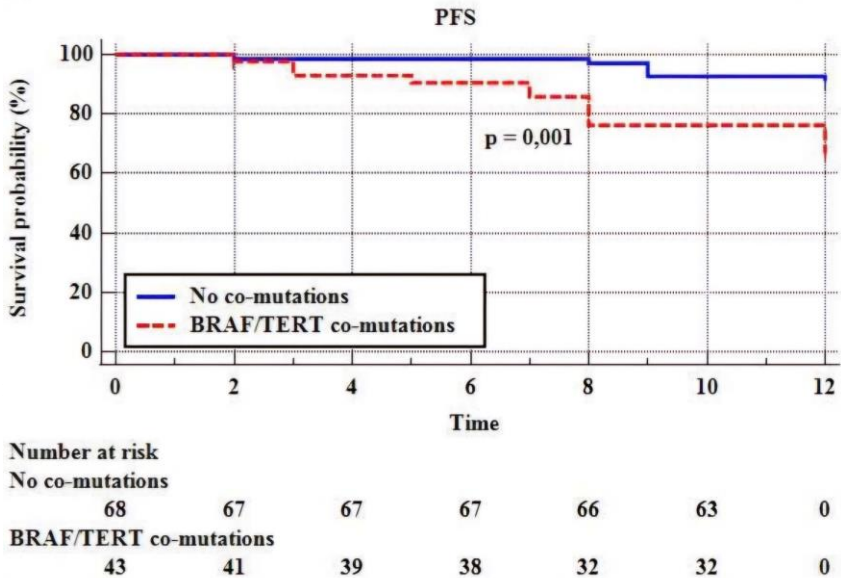


Figure 3.9. Kaplan–Meier curve comparing progression-free survival according to BRAF/TERT co-mutation status

Comment:

Progression-free survival (PFS) after reoperation in patients with radioiodine-refractory differentiated thyroid carcinoma (RAI-R DTC) harboring BRAF/TERT co-mutations (10.6 months) was significantly shorter than that in patients without co-mutations (11.7 months), with $p = 0.001 < 0.005$.

Chapter 4. DISCUSSION

4.1. Observations on clinical features, histopathology, and the prevalence of *BRAF* V600E and *TERT* promoter mutations in RAIR-DTC patients

4.1.1. General characteristics of recurrent cervical differentiated thyroid carcinoma

4.1.4.2. Association between *BRAF* V600E and *TERT* promoter mutations and radioiodine uptake in recurrent cervical lesions

Our results indicate that *TERT* promoter mutations and *BRAF/TERT* co-mutations are associated with an increased risk of loss of I-131 avidity in DTC patients (Table 3.12). These findings are consistent with Liu J. et al. (2019), who studied 103 patients with recurrent radioiodine-refractory papillary thyroid carcinoma and genotyping of primary tumor tissue, reporting that 70.3%, 55.6%, and 97.4% of patients harbored *BRAF* V600E only, *TERT* only, and *BRAF/TERT* co-mutations, respectively, compared with 30.2% of patients with no mutations. Synergistic analysis of the two genes showed that the combination of *BRAF* V600E and *TERT* promoter mutations increased the risk by 13.28-fold (95% CI: 1.54–114.46; $p = 0.019$). Our study confirms that *BRAF/TERT* co-mutations have the strongest impact on loss of RAI avidity in RAIR-DTC. Both our study and Liu et al. demonstrate that the co-mutation affects metastatic progression in DTC.

4.2. Analysis of the relationship between selected clinical features, histopathology, treatment outcomes, and mutation/co-mutation status of *BRAF* V600E and *TERT* promoter in RAIR-DTC

4.2.1. Association between clinical features, histopathology, treatment outcomes, and BRAF V600E/TERT mutation status

4.2.1.2. Comparison of characteristics by mutation status in RAIR-DTC patients

Our results show that mean age at diagnosis, proportion of patients ≥ 55 years at recurrence, T4 tumor size, high recurrence risk, pre I-131 stimulated Tg, and suppressed Tg at recurrence were significantly higher in RAIR-DTC patients with *BRAF/TERT* co-mutations compared with patients without co-mutations (Tables 3.15 and 3.16). Park J. et al. (2021) reported that *TERT* mutation is an independent prognostic factor for thyroid cancer mortality, detected in 10.9% (43/393) of differentiated thyroid carcinomas (9.8% papillary and 16.7% follicular), and associated with age >55 years, extrathyroidal extension, distant metastasis, and stage III–IV at diagnosis ($p < 0.001$). Our study similarly found that *TERT* mutations were strongly associated with clinical features indicative of progressive disease, including higher mean age at diagnosis and recurrence, male sex, T4 tumor size, high recurrence risk, elevated pre I-131 stimulated Tg, and elevated suppressed Tg at recurrence (Table 3.16).

4.2.1.4. Comparison of histopathological characteristics of recurrent cervical lesions by BRAF/TERT co-mutation status

Yang X. et al. (2017) analyzed 10 patients with *BRAF/TERT* co-mutations (4 conventional PTC, 3 papillary–follicular variants, 2 diffuse sclerosing variants, 1 tall cell variant) versus 56 patients without co-mutations, finding no significant difference in

histopathology, possibly due to small sample size. Our findings are consistent: there were no marked differences in recurrent lymph node histopathology between co-mutation and no co-mutation groups (Table 3.18). Cervical lymph nodes/recurrent lesions in the co-mutation group did not differ significantly from the no co-mutation group, but there was a trend toward higher rates of necrosis, muscle invasion, perinodal soft tissue invasion, and perineural invasion in the co-mutation group (Table 3.19).

4.2.1.8. Cox regression analysis of clinical factors and gene mutations with disease progression after reoperation

In our study, multivariate analysis identified male sex, extracervical metastases, and *BRAF/TERT* co-mutations as independent prognostic factors increasing the risk of progression after cervical lymph node dissection in RAIR-DTC patients (Table 3.23). *TERT* promoter mutations can activate telomerase, contributing to cellular immortality, increased risk of progression, and resistance to I-131 therapy in differentiated thyroid carcinoma. Studies consistently show that the coexistence of *BRAF* V600E and *TERT* promoter mutations markedly increases the risk of early recurrence in RAIR-DTC, correlating with poor prognosis. The presence of these co-mutations in tumor samples can be used for high-risk stratification to guide tailored therapeutic strategies.

4.2.2. Prognostic value of selected clinical features, histopathology, treatment outcomes, and BRAF V600E/TERT mutation/co-mutation status in RAIR-DTC

4.2.2.5. Comparison of progression-free survival after reoperation

according to histopathological type

Histopathological type is also a determinant of survival in DTC. Patients with non-conventional papillary variants often exhibit recurrence or distant metastasis and may require aggressive management, including surgery, radioiodine therapy, or systemic tyrosine kinase inhibitors. Chrisoulidou A. et al. (2011) reported that aggressive papillary variants increased the risk of death 4.56-fold compared with conventional PTC. In our study, the small number of patients with aggressive variants and limited follow-up likely contributed to the lack of statistical significance (Figure 3.6). Further studies with larger cohorts and longer follow-up are needed for robust conclusions.

4.2.2.8. Comparison of progression-free survival after reoperation according to BRAF/TERT mutation status

Kaplan–Meier analysis of PFS after reoperation showed that RAIR-DTC patients with *BRAF/TERT* co-mutations had significantly shorter PFS than those without co-mutations (Figure 3.9). Al-Masri M. et al. (2021) studied 128 PTC patients with a 71% *BRAF* V600E mutation rate and found that *BRAF* V600E alone did not affect recurrence/metastasis rate, PFS, or overall survival. Our study similarly found no significant difference in PFS between *BRAF*-positive and *BRAF*-negative groups.

CONCLUSIONS

Based on the analysis of clinical features, histopathology, and *BRAF* V600E and *TERT* promoter mutations in 111 patients with recurrent cervical differentiated thyroid carcinoma refractory to I-131, we draw the following conclusions:

1. Observations on clinical features, histopathology, and mutation prevalence in RAIR-DTC patients

- The mean age at initial diagnosis in the I-131 refractory group was 44.9 ± 13.8 years. The median cumulative I-131 dose was 280 mCi (range: 225–375 mCi), and 9% of patients received a total I-131 dose ≥ 600 mCi. The median time to cervical recurrence refractory to I-131 was 29 months.

- Aggressive papillary variants in recurrent cervical lesions accounted for 5.4% in RAIR-DTC patients: 2.7% tall cell variant, 0.9% diffuse sclerosing variant, 0.9% columnar cell variant, and 0.9% hobnail variant.

- Among RAIR-DTC patients with recurrent cervical lesions: 38.7% had *BRAF/TERT* co-mutations.

- The presence of *BRAF/TERT* co-mutations increased the risk of I-131 resistance 4.8-fold (OR = 4.8; 95% CI: 1.2–19.9; $p = 0.009$).

2. Relationship between clinical features, histopathology, treatment outcomes, and *BRAF/TERT* mutation status

- Patient age at initial diagnosis and at recurrence, T4 tumor size, and higher Tg values were statistically significantly greater in the *BRAF/TERT* co-mutation group compared with the group without

co-mutations.

- The proportion of aggressive papillary variants (tall cell, columnar cell, hobnail, diffuse sclerosing) tended to be higher in the *BRAF/TERT* co-mutation group than in patients without co-mutations.

- Clinical factors associated with the presence of *BRAF/TERT* co-mutations in recurrent lesions included age >55 years, T4 tumor, and high recurrence risk.

- Progression after reoperation was significantly higher in male patients, age ≥ 55 years, elevated Tg at recurrence, and those with *BRAF/TERT* co-mutations ($p < 0.05$).

- In univariate analysis, age >55 at recurrence, male sex, distant metastasis, suppressed Tg at recurrence, and *BRAF/TERT* co-mutations were associated with disease progression.

- Multivariate analysis identified male sex, distant metastasis, and *BRAF/TERT* co-mutations as independent prognostic factors for progression after cervical reoperation in RAIR-DTC patients.

- Progression-free survival (PFS) was significantly shorter in patients aged ≥ 55 , male patients, those with T4 tumors, and patients with *BRAF/TERT* co-mutations.