Original Article

BRAF V600E mutation co-existing with oncogenic mutations is associated with aggressive clinicopathologic features and poor prognosis in papillary thyroid carcinoma

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ABSTRACT

Background: The aim of this study was to evaluate the correlation among mutations in cancer-related genes, clinicopathologic features, and clinical outcome in classical papillary thyroid carcinoma (PTC).
Patients and methods: A total of 130 patients with classical PTC who underwent curative surgery between April 2012 and June 2023 at Hokuto Hospital were included. Mutations in targeted regions of 160 cancer-related genes were detected by next-generation sequencing (NGS)-based cancer panel testing.
Results: The BRAF V600E mutation was detected in 108 (83.1%) of 130 PTC patients. Among the 108 patients with the BRAF V600E mutation, other co-existing oncogenic mutations were found in 12 (9.2%) patients. When we divided into 3 groups of no mutations, BRAF V600E mutation alone, and BRAF V600E and other oncogenic mutations, significant differences were observed in terms of tracheal invasion (P = 0.0024), and bilateral neck lymph node metastasis (P = 0.0047). Kaplan–Meier analysis of overall survival (OS) revealed patients with BRAF V600E and other oncogenic mutations had significantly poorer survival than those with BRAF V600E mutation alone (P = 0.0026). Multivariate cox proportional hazard analysis revealed BRAF V600E and other oncogenic mutations was an independent prognostic factor for OS (HR: 10.559; 95%CI: 1.007-110.656, P = 0.0493).
Conclusions: The BRAF V600E mutation co-existing with other oncogenic mutations but not the BRAF V600E mutation alone was associated with aggressive clinicopathologic features, resulting in poor prognosis in patients with classical PTC. Detection of oncogenic mutations using NGS-based cancer panel testing could enhance understanding of the clinical features of classical PTC.

1. Introduction

Prognosis of patients with papillary thyroid carcinoma (PTC) is generally favorable,[1–3] however, some patients with PTC show aggressive features, such as invasion to the adjacent structures, multiple neck lymph node metastasis, and distant metastasis at the first visit. Next-generation sequencing (NGS)-based cancer panel testing has been used to assist histologic diagnosis and it seems to determinate the adaptation of molecular-targeted agents, as well as for predicting clinical outcome in human carcinomas,[4] however, the testing has not been performed for PTC in clinical settings. v-raf murine sarcoma viral oncogene homolog B1 (BRAF), a serine threonine kinase and downstream signaling molecule of RAS, is a potent activator of the MAPK signaling pathway.[1] Frequency of

BRAF V600E in patients with PTC in East Asian countries reportedly ranges from 71 to 83%,[5–10] which is higher than that in the United States and Europe reportedly ranges from 29 to 69%.[1,11–15] Association between the *BRAF* V600E mutation alone, aggressive clinicopathologic features and prognosis in patients with PTC is still controversial.[9,15–18] In the present study, we performed NGS-based cancer panel testing for PTC and examined the correlations between identified oncogenic mutations, and clinicopathologic features and prognosis during the long-term observation.

2. Patients and Methods

2.1. Patients

The study group consisted of 130 Japanese patients (99 females and 31 males) who underwent curative surgery between April 2012 and June 2023 at Hokuto Hospital, Hokkaido, Japan. Patients under 20 years of age with classical PTC, those with PTC variants, and those with distant metastasis at surgery were excluded in this study. PDTC patients were excluded based on the criteria of the Turin proposal, which is also endorsed by the WHO classification system.[19] Clinicopathologic data for each patient were retrospectively reviewed, and data for some of the patients have reported elsewhere.[9] Tumor size was measured by ultrasonography, and serum anti-thyroid peroxidase (TPO)/thyroglobulin antibody levels were measured before surgery. Surgical treatments were conducted in accordance with guidelines on the management of thyroid tumors by the Japan associations of endocrine surgeons.[20] Briefly, Patients with T1a, T1b, and T2 and those with T3a, T3b and T4a were treated with hemi-thyroidectomy and total thyroidectomy, respectively. Patients with N0 and N1a and those with N1b were treated with central compartment dissection and lateral neck dissection, respectively. Pathologic (p)T, pN, pStage, multifocality of carcinoma in the thyroid gland, presence of histologic poorly differentiated components such as solid, trabecular and insular components, strap muscle invasion, recurrent nerve invasion, trachea invasion, and lymph node metastasis were examined histologically using surgical specimens. Histologic diagnosis was performed by two experienced pathologists. Patients were classified according to the 8th edition of the AJCC/TNM staging system.[21]

2.2. NGS-based cancer panel testing

Surgical specimens were obtained from 130 patients with classical PTC who underwent thyroidectomy. Genetic analysis was performed as previously described.[9] Briefly, total DNA was extracted from 5-μm-thick formalin-fixed paraffin-embedded (FFPE) tissue sections of PTC specimens using a Maxwell 16 FFPE Plus LEV DNA purification kit (Promega, Madison, WI). The quality of genomic DNA was assessed using a Qubit dsDNA BR assay kit (Life Technologies, Carlsbad, CA) and a GeneRead DNA QuantiMIZE assay kit (Qiagen, Valencia, CA). Amplicon sequencing of targeted regions of 160 cancer-related genes (Table S1) was carried out using the GeneRead DNAseq Targeted Panels V2 Human Clinically Relevant Tumor Panel (NGHS-101X; Qiagen). Library quality was assessed using an Agilent 2100 bioanalyzer (Agilent, Santa Clara, CA) and GeneRead Library Quant kit (Qiagen). The libraries were sequenced using an Illumina MiSeq platform (Illumina, San Diego, CA). Raw read data obtained from the amplicon sequencing were processed using online analytical resources from the GeneRead DNAseq Variant Calling Service for analysis of mutations. Actionable mutations were detected.

2.3. Statistical analysis

Results are expressed in the form of number (%) or mean ± standard deviation. Associations between groups were determined using Pearson's chi-square test for categorical variables and Kruskal–Wallis and Scheffe's tests for continuous variables. Overall survival (OS) rates were calculated using the Kaplan–Meier method and compared using the log-rank test. Time was defined as the period starting from the date of surgery to the date of death by any cause for OS. Cox proportional hazard modeling were used to identify the factors associated with OS. A p-value of <0.05 was considered indicative of statistical significance. BellCurve for Excel (Social Survey Research Information, Tokyo, Japan) statistical software was used for all analyses.

3. Results

3.1. Clinicopathologic features

In 130 patients with classical PTC, mean age was 59.2 ± 14.8 years (Table 1). Primary tumor size was 18.8 ± 10.5 mm. High levels of serum anti-TPO and/or anti-thyroglobulin antibodies were detected in. 27 patients (21%) Hemi-thyroidectomy with routine central compartment dissection and those with lateral neck dissection were performed in 80 (62%) and 9 (7%) patients, respectively. Total thyroidectomy with routine central compartment dissection and those with lateral neck dissection were performed in 23 (18%) and 18 (14%) patients, respectively. Disease pathologic T classification was pT1a in 39 (30%) patients, pT1b in 29 (22%), pT2 in 12 (9%), pT3a in 5 (4%), pT3b in 24 (18%), and pT4a in 21 (16%). The pathologic N classification was pN0 in 60 (46%) patients, pN1a in 45 (35%), and pN1b in 25 (19%). pStage classification was pStage I in 71 (54%) patients, pStage II in 44 (34%), and pStage III in 15 (12%). Histologically, multifocal tumor lesions in the thyroid were observed in 69 patients (53%). Poorly differentiated components were observed in 5 patients (4%). Invasion to strap muscle, recurrent nerve, and trachea was observed in 35 patients (27%), 20 (15%) and 8 (6%), respectively. Bilateral neck lymph node metastasis was observed in 5 patients (4%). Follow-up period was 52.0 ± 49.3 months. One hundred twenty-five (96%) of the patients were alive without thyroid carcinoma. Three patients (3%) died with local recurrence after surgery, 2 times of radioactive iodine (RAI) with I-131 150 mCi treatment, and post-recurrence lenvatinib administration. The remaining 2 patients (2%) died of other causes. The 10-year OS rate among the 130 patients was 87.6%.

3.2. Mutational analysis

The *BRAF* V600E mutation was present in 108 (83.1%) of the 130 patients with classical PTC. No actional mutations, including in *BRAF,* were identified in the other 22 (16.9%) patients with PTC. Among the 108 patients with the *BRAF* V600E mutation, other co-existing oncogenic mutations were found in 12 (9.2%) patients (Table 2). Among the 12 patients with the *BRAF* V600E mutation and other oncogenic mutations, mutations in genes of the PIK3/Akt/mTOR signaling pathway were identified in 7 patients (6 patients with mutations in *PIK3CA* and 1 with *PTEN* mutations). No patients with oncogenic mutations and without *BRAF* V600E mutation were not seen. Therefore, patients could be divided into three groups: patients with no actional mutations (Group I, n = 22), patients with the *BRAF* V600E mutation alone (Group II, n = 96), and patients with the *BRAF* V600E and other oncogenic mutations (Group III, n = 12).

Table 1
Clinicopathologic characteristics of patients with papillary thyroid carcinoma according to mutational status.

Variable		Total (n = 130)	Group I: No mutations (n = 22)	Group II: BRAFV600E alone (n = 96)	Group III: *BRAF*V600E + other oncogenic mutations (n = 12)	P-value
Sex	female	99 (76%)	19 (86%)	71 (74%)	9 (75%)	0.6341
	male	31 (24%)	3 (14%)	25 (26%)	3 (25%)	
Age at surgery (years)		59.2 ± 14.8	58.7 ± 14.5	60.1 ± 14.5	56.9 ± 21.1	0.6892
anti-TPO/thyroglobulin antibody	normal	103 (69%)	13 (59%)	80 (83%)	10 (83%)	0.0691
	high	27 (21%)	9 (41%)	16 (17%)	2 (17%)	
Tumor size (mm)		18.8 ± 10.5	18.6 ± 9.2	18.3 ± 15.6	18.4 ± 9.8	0.9989
pT	1a-3b	110 (85%)	19 (86%)	83 (86%)	8 (67%)	0.8207
	4a	20 (15%)	3 (14%)	13 (14%)	4 (33%)	
pN	0, 1a	106 (82%)	17 (73%)	81 (83%)	8 (67%)	0.5939
	1b	24 (18%)	5 (23%)	15 (17%)	4 (33%)	
pStage	I, II	115 (88%)	21 (95%)	85 (89%)	9 (75%)	0.4722
	III	15 (12%)	1 (5%)	11 (11%)	3 (25%)	
Multifocality	−	61 (47%)	7 (32%)	51 (53%)	3 (25%)	0.1918
	+	69 (53%)	15 (68%)	45 (47%)	9 (75%)	
Poorly differentiated components	−	125 (96%)	22 (100%)	93 (97%)	10 (83%)	0.3737
	+	5 (4%)	0 (0%)	3 (3%)	2 (17%)	
Strap muscle invasion	−	95 (73%)	21 (95%)	66 (69%)	8 (67%)	0.0722
	+	35 (27%)	1 (5%)	30 (31%)	4 (33%)	
Recurrent nerve invasion	−	110 (85%)	19 (86%)	84 (85%)	9 (75%)	0.8696
	+	20 (15%)	3 (14%)	14 (15%)	3 (25%)	
Trachea invasion	−	122 (94%)	21 (95%)	93 (97%)	8 (67%)	0.0024*
	+	8 (6%)	1 (5%)	3 (3%)	4 (33%)	(Group II vs. III $P < 0.001$*)
Bilateral neck lymph node metastasis	−	125 (96%)	21 (95%)	95 (99%)	9 (75%)	0.0047*
	+	5 (4%)	1 (5%)	1 (1%)	3 (25%)	(Group II vs. III $P < 0.001$*)
Thyroidectomy	hemi-	88 (68%)	15 (68%)	66 (69%)	7 (58%)	0.8878
	total	42 (32%)	7 (32%)	30 (31%)	5 (42%)	
Lymph node dissection	central compartment	103 (79%)	16 (73%)	79 (82%)	8 (67%)	0.5684
	lateral neck	27 (21%)	6 (27%)	17 (18%)	4 (33%)	
Follow-up period (months)		52.0 ± 49.3	43.5 ± 39.7	52.8 ± 42.5	61.6 ± 44.3	0.7124
Clinical outcome	Alive without PTC	125 (96%)	22 (100%)	94 (98%)	9 (75%)	
	Dead with recurrence	3 (2%)	0 (0%)	1 (1%)	2 (17%)	0.1615
	Died, other causes	2 (2%)	0 (0%)	1 (1%)	1 (8%)	

−, not present; +, present; Data are shown as number (%) or mean ± SD.; Pearson's chi-square test was used for categorical variables. Kruskal−Wallis and Scheffe's tests were used for continuous variables.; *P < 0.05 was considered statistically significant.

Table 2
Grouping in 130 patients with papillary thyroid carcinoma according to mutational status and clinical characteristics of 12 patients with BRAF V600E and other oncogenic mutations.

Group	n	Mutational status	Age (years)/Sex	pT	pN	pStage	Treatment	Outcome (months)
Group I	22	No mutations						
Group II	96	*BRAF* V600E alone						
		BRAF V600E + other oncogenic mutations						
		PIK3CA_delPV104P	77/F[a]	4a	0	III	H, C	alive (96)
		PIK3CA_D350Y	37/F	1b	1a	I	T, C	alive (109)
		PIK3CA_D1045E	49/F	3b	1a	I	H, C	alive (122)
		PIK3CA_H1047L	77/M	4a	1b	III	T, L, RAI, lenvatinib	DLM (74)
		PIK3CA_H1047R	28/M	1a	1b	I	H, L	alive (115)
Group III	12	PIK3CA_A1046T, TP53_R306*, FGFR3_G382R	77/F[a]	4a	1b	III	T, L	DOC (52)
		PTEN_c.1026+2T > G	82/F	1a	1a	II	T, C	alive (14)
		TP53_S171R	42/F	1a	0	I	H, C	alive (119)
		GRIN2A_L649W	44/M	4a	1b	I	T, L, RAI, lenvatinib	DL (19)
		CREBBP_P1946A	54/F	2	0	I	H, C	alive (7)
		EP300_S285Ifs*47	49/F	1b	1a	I	H, C	alive (5)
		RET_Y806C	76/F	1b	0	I	H, C	alive (7)

H: hemi-thyroidectomy; T: total thyroidectomy; C: central compartment dissection; L: lateral neck dissection; RAI: radioactive iodine; DLM: Dead with local recurrence and metastasis; DOC: Died, other causes; DL: Dead with local recurrence.
[a] Data had been described elsewhere 9.

3.3. Mutational analysis and clinicopathologic features

There were no statistically significant differences in clinicopathologic features between patients with no mutations (Group I, n = 22) and those with the *BRAF* V600E mutation (Group II and Group III, n = 108) (data not shown). Significant differences were observed between the three groups in terms of tracheal invasion (P = 0.0024), and bilateral neck lymph node metastasis (P = 0.0047) (Table 1). No significant differences were observed between the three groups in terms of sex, age at surgery, serum anti-TPO/thyroglobulin antibodies, tumor size, pT, pN, pStage, multifocality, presence of poorly differentiated components, strap muscle invasion, recurrent nerve invasion, treatment methods including thyroidectomy and neck dissection, follow-up period, or clinical outcomes.

3.4. Mutational analysis and clinical outcome

The 10-year OS rates of patients with no mutations (Group I), *BRAF* V600E mutation alone (Group II), and *BRAF* V600E and other oncogenic mutations (Group III) were 100%, 90%, and 62.5%, respectively (Fig. 1). Kaplan—Meier analysis of OS showed Group III had significantly poorer survival than Group II (P = 0.0026). Univariate cox proportional hazard analysis revealed that pNb (P = 0.0201), pStage (P = 0.0396), presence of poorly differentiated components (P = 0.0047), tracheal invasion (P = 0.0018), bilateral neck lymph node metastasis (P = 0.0074), total thyroidectomy with lateral neck dissection (P = 0.0059), and Group III (P = 0.0080) was significantly associated with poorer OS (Table 3). Multivariate cox proportional hazard analysis revealed that Group III was an independent prognostic factor for OS (HR: 10.559; 95%CI: 1.007—110.656, P = 0.0493) (Table 4).

4. Discussion

Over the last decade, understanding of the genomic landscape that defines PTC has increased dramatically. *BRAF* V600E is the most common oncogenic mutation in patients with PTC.[22] The *BRAF* V600E mutation markedly increases the kinase activity of BRAF by evoking a 480-fold increase in phosphorylation of ERK1/2
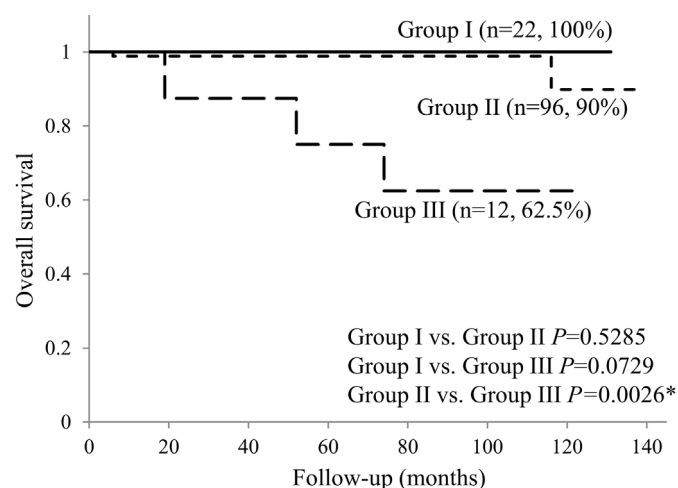
compared with wild-type BRAF, resulting in the expression of a number of genes that are involved in cell proliferation, tumorigenesis, dedifferentiation, survival, and promotion of epithelial—mesenchymal transition.[23] *BRAF* V600E-mutant thyroid carcinomas tend to exhibit higher MAPK output than their *RET*-driven counterparts due to differences in negative feedback signals.[24]

Age, tumor size, extra-thyroidal extension, histologic tumor differentiation, lymph node metastasis, and distant metastasis in patients with PTC have been shown as prognostic factors of predicting recurrence and death by American Thyroid Association (ATA) guidelines[25] and the AJCC/TNM staging system.[21] Results in the present study revealed that pN, pStage, presence of poorly differentiation component, tracheal invasion, and bilateral node metastasis were significantly associated with poor prognosis. The 10-year OS rate among the 130 patients was 87.6%. Therefore, our patient's cohort and treatment outcome were thought to be comparable to other studies.[20] It was suitable for further analysis.

In the present study, the frequency of the *BRAF* V600E mutation in patients with classical PTC was 83.1%, which was higher than the previously reported range of 29—69% in the United States and Europe.[1,11—15] The higher frequency in the present study could be attributed to tumors in patients from specific geographic locations and differences in methodology. Residents in eastern Asia commonly consume iodine-containing seaweed as part of their regular diet. The region in which our hospital is located, and in which all of the patients involved in this study resided, Hokkaido, Japan, is a well-known for seaweed (e.g., Kombu [kelp]) production area and regarded as an area of high iodine intake.[26] Iodine intake is reportedly linked to a higher frequency of *BRAF* mutations in patients with PTC.[27—29] These reports are consistent with the average frequency of *BRAF* V600E mutation, which is higher in patients with PTC in the iodine-sufficient countries such as Japan, South Korea, and China (76%) than in patients in the iodine-deficient areas of south and central Asia (45%).[10]

The high frequency of the *BRAF* V600E mutation in the present study (83.1%) compared with other studies, in which the frequency varies widely between 27% and 82% in patients with PTC in Japan, could be related to the use of NGS in this study.[18,30—37] The genes of patients in these previous studies were analyzed using Sanger gene sequencing (SGS). In the present study, we conducted NGS on an Illumina Miseq sequencer to analyze FFPE tissue sections obtained from 130 patients with PTC. In general, the detection sensitivity of NGS reported in previous studies is >94%, which is superior to that of SGS.[38] NGS methods enables the analysis of somatic mutation using a small amount of tumor-specific DNA.[39] Moreover, targeted NGS is more rapid and cost effective than SGS.[40] NGS-based cancer panel testing for human carcinomas, including PTC should be widely available and routinely applied in clinical settings to assist in risk assessment and to inform the selection of molecular-targeted agents for patients with advanced-stage PTC.

With regard to the *BRAF* V600E mutation and clinicopathologic features and clinical outcomes in patients with PTC, numerous reports and multicenter studies have described significant correlations between the *BRAF* V600E mutation and advanced stages of disease, as well as increased risk of lymph node involvement, disease recurrence, distant metastasis, and poor prognosis.[15,16,28,30,35,41—44] A retrospective analysis of patients with PTC found a mortality rate of 5.3% in patient with *BRAF* V600E mutation versus 1.1% in patients without the *BRAF* V600E mutation.[45] However, these observations were not confirmed in whole or in part by other studies.[17,18,36,37,46—54] In the present study, no correlations between clinicopathologic features and prognosis were identified in the 108 PTC patients with the *BRAF* V600E mutation and 22 PTC patients without the *BRAF* V600E mutation. There

Fig. 1. Kaplan—Meier analysis of overall survival in patients with classical papillary thyroid carcinoma according to mutational status. The 10-year overall survival rates of patients with no mutations (Group I), *BRAF* V600E mutation alone (Group II), and *BRAF* V600E and other oncogenic mutations (Group III) were 100%, 90%, and 62.5%, respectively. Group III had significantly poorer survival than Group II (log-rank test, P = 0.0026).

Table 3
Univariate cox proportional hazards analysis of overall survival in patients with papillary thyroid carcinoma.

Variable		n	HR (95%CI)	P-value
Sex	Female	99	0.368 (0.060—2.246)	0.2789
	Male	31		
Age at surgery (years)	<55	55	2.780 (0.305—25.346)	0.3646
	≥55	75		
anti-TPO/thyroglobulin antibody	normal	103	0.635 (0.051—6.227)	0.6532
	high	27		
Tumor size (mm)	<20	91	9.272 (0.984—83.142)	0.0567
	≥20	39		
pT	1a-3b	110	4.725 (0.770—27.869)	0.1071
	4a	20		
pN	0, 1a	106	13.502 (1.504—121.209)	0.0201*
	1b	24		
pStage	I, II	115	6.620 (1.093—40.065)	0.0396*
	III	15		
Multifocality	−	61	4.487 (0.3848—31.589)	0.2667
	+	69		
Poorly differentiated components	−	125	17.125 (2.388—122.765)	0.0047*
	+	5		
Strap muscle invasion	−	95	3.385 (0.547—20.914)	0.1895
	+	35		
Recurrent nerve invasion	−	110	11.160 (0.950—186.973)	0.0601
	+	20		
Trachea invasion	−	122	17.381 (2.886—104.686)	0.0018*
	+	8		
Bilateral neck lymph node metastasis	−	125	11,821 (1.940—72.040)	0.0074*
	+	5		
Surgical methods	H + C, H + L, T + C	112	21.750 (2.425—195.055)	0.0059*
	T + L	18		
Mutational status	Group I + II	118	11.446 (1.890—69.332)	0.0080*
	Group III	12		

−, not present; +, present; H: hemi-thyroidectomy; T: total thyroidectomy; C: central compartment dissection; L: lateral neck dissection; HR: hazard ratio; CI: confidence interval; *P < 0.05 was considered statistically significant.

Table 4
Multivariate cox proportional hazards analysis of overall survival in patients with papillary thyroid carcinoma.

Variable		n	HR (95%CI)	P-value
Sex	Female	99	0.383 (0.054—2.702)	0.3355
	Male	31		
Age at surgery (years)	<55	55	3.810 (0.134—108.139)	0.4333
	≥55	75		
pStage	I, II	114	2.122 (0.133—33.827)	0.5943
	III	16		
Mutational status	Group I + II	118	10.559 (1.007—110.656)	0.0493*
	Group III	12		

HR: hazard ratio; CI: confidence interval; *P < 0.05 was considered statistically significant.

is some uncertainty regarding the prognostic value of the *BRAF* V600E mutation, as this mutation was found in a large proportion of PTC patients in the present study (83.1%) who exhibited a good prognosis, with a 10-year OS rates of 87.6%. *BRAF* V600E mutation is often identified in papillary microcarcinomas without neck lymph node metastasis. Therefore, we conclude that the *BRAF* V600E mutation alone was not associated with aggressive clinicopathologic features or poor prognosis in the present study.

Among the 130 patients with classical PTC in the present study, mutations in genes of the PI3K/Akt/mTOR signaling pathway were identified in 7 (5%) patients, including 6 patients with *PIK3CA* mutations and 1 (1%) with *PTEN* mutations. The frequency of *PIK3CA* mutations in patients with PTC reportedly ranges from 3% to 11%.[6,55—57] Over 90% of mutations in the *PIK3CA* gene in human carcinomas occur in 4 regions: the p85-binding (exons 1 and 2), C2 (exon 7), helical (exon 9), and catalytic (exon 20) domains.[58] The 6 mutations in *PIK3CA* we identified were located within these regions. *PIK3CA* mutations are related to tumor development, progression, and more aggressive features in patients with PTC.[57,59—61] Co-existing *PIK3CA* mutations were identified in 6 (6%) of the 108

patients with the *BRAF* V600E mutation in the present study. The frequency of co-existing *BRAF* V600E and *PIK3CA* mutations in patients with PTC reportedly ranges from 2 to 8.3%.[6,56,57,61] In an analysis of the mutational landscape of 139 patients with advanced PTC, Chen et al reported that the rate of co-existing *BRAF* V600E mutation with oncogenic mutations such as *PIK3CA* and *TP53* mutations was significantly higher in patients with advanced-stage PTC (7%) compared with all PTC patients in The Cancer Genome Atlas cohort (2.5%).[57] The cooperation and synergistic activation of both the MAPK and PI3K/Akt/mTOR signaling pathways caused by the *BRAF* V600E mutation and mutations in the PI3K/Akt/mTOR signaling pathway, respectively, resulted in increased proliferation and survival of tumor cells and more aggressive clinicopathologic features.[62,63] In addition, a thyroid-specific *BRAF* V600E murine PTC model progressed to lethal ATC as a result of either the activating *PIK3CA* H1047R mutation or inactivating *PTEN* mutations.[64]

Other than *PIK3CA*, *PTEN* and *TP53* mutations, some oncogenic mutations with *BRAF* V600E mutation were identified. *GRIN2A* encodes a glutamate N-methyl-D-aspartic acid receptor subunit ε-1 that is part of the class of ionotropic glutamate receptors and bears

the agonist binding site for glutamate.[65] Somatic mutation of *GRIN2A* results in dominant negative effects inhibiting the tumor suppressive phenotype of wild type *GRIN2A*. The somatic mutation in *GRIN2A* has been reported in various solid carcinomas, including melanoma, lung, and colorectal carcinoma.[66] In the present study, a patient with co-existence of *BRAF* V600E and *GRIN2A* L649W mutations had aggressive features such as tracheal invasion and bilateral neck lymph node metastasis at the first visit and died with local recurrence after total thyroidectomy and bilateral neck dissection, and RAI and lenvatinib administration. The acetyl-transferase CREB-binding protein (CREBBP) and E1A-binding protein P300 (EP300) are two closely related regulators among the histone acetyltransferases family. Both are widely expressed within and outside the hematopoietic system and serve as tumor activators or suppressors depending on the situation.[67] *CREBBP* and *EP300* regulate various key physiological functions, including cell apoptosis, proliferation and differentiation, DNA repair and somatic cell reprogramming.[67] Somatic mutations of *CREBBP* and *EP300* were reportedly associated with tumorigenesis and the progression of hematological malignancies, colorectal carcinoma,[68] and head and neck squamous cell carcinoma.[69] The consequences of the mutations of *CREBBP* and *EP300* in patients with PTC are still unknown. Further investigations would be needed. Recent studies have revealed that co-existing *BRAF* V600E and *TERT* promoter mutations was identified in 6.9%–10.1% patients with PTC and associated with more aggressive features and poor prognosis.[14,52] In the present study, we used a commercially available cancer panel testing 160 cancer-related genes commonly associated with human carcinomas for our NGS analysis, but this panel did not include the *TERT* promoter. Overall, the co-existence of *BRAF* V600E and other oncogenic mutations may ultimately prove to be a more specific and important prognostic marker for patients with PTC as shown by ATA guidelines.[25]

Major limitation of the present study was small sample size. We analyzed only 130 patients with PTC at a single institution, which might be an insufficient number of patients to correlate mutational status with clinical significance. Moreover, a small number of patients with variety of mutational patterns was lumped together into the same group (Group III). To overcome the limitation multicenter analysis will be needed. In conclusion, NGS-based cancer panel testing of 160 cancer-related genes in 130 patients with classical PTC revealed a high frequency of the *BRAF* V600E mutation (83.1%). Patients harboring the *BRAF* V600E mutation along with other oncogenic mutations had more aggressive clinicopathologic features such as tracheal invasion and lateral neck lymph node metastasis, resulting in poor prognosis. Therefore, NGS-based cancer panel testing can enhance our understanding of clinicopathologic features and prognostic outcomes in patients with PTC.

Authors' contributions

NB, TG, AK, SSa, RT, SH, and MH performed surgeries and provided bedside care. SB and TI performed mutational analyses. YK and HN confirmed the mutational analysis data and histologic diagnoses. NB analyzed the clinical data and drafted the manuscript. HK conceived the study design. All authors approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.asjsur.2023.09.049.

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