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ORIGINAL ARTICLE

Molecular Profile and Clinicopathologic Features of Follicular Variant Papillary Thyroid Carcinoma

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Abstract

The non-invasive encapsulated follicular variant of papillary thyroid carcinoma (FVPTC) has an indolent clinical behavior. Recently, it was proposed that this tumor type should be reclassified as non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). To characterize NIFTPs, we evaluated the molecular and clinicopathologic characteristics of each FVPTC subtype. This study enrolled 29 patients with FVPTC who underwent thyroidectomy between January 2007 and June 2017. They were classified as non-invasive encapsulated FVPTC (NIFTP, $n = 10$), invasive encapsulated FVPTC ($n = 11$), and infiltrative FVPTC ($n = 8$) by two independent pathologists. Genetic alterations were analyzed by targeted next-generation sequencing using formalin-fixed, paraffin-embedded tissue samples and the clinicopathologic characteristics were retrospectively reviewed. There was no difference in preoperative cytologic classification between NIFTPs and invasive encapsulated FVPTCs, whereas infiltrative FVPTC was more likely to be Bethesda class VI than the encapsulated type (50% versus 9.5%; $P = 0.033$). Lymph node metastasis was not found in NIFTPs. There was no $BRAF^{V600E}$ mutation in NIFTPs, whereas one of 11 invasive encapsulated FVPTCs and three of 8 infiltrative FVPTCs harbored $BRAF^{V600E}$. *RAS* mutations were frequently detected in encapsulated FVPTCs (5 of 10 NIFTPs and 4 of 11 invasive encapsulated FVPTCs) but were only detected in one case of the infiltrative type. There were no differences in molecular or clinicopathologic profiles between non-invasive and invasive encapsulated FVPTCs, except for lymph node metastasis and the presence of $BRAF^{V600E}$. NIFTP has favorable pathologic characteristics with a high frequency of *RAS* mutations.

Keywords NIFTP · *BRAF* · *RAS* · Thyroid neoplasm

Introduction

The papillary thyroid carcinoma (PTC) family of tumor is defined histologically by the presence of clear, overlapping nuclei with grooves and pseudoinclusions [1]. The follicular variant of PTC (FVPTC) is the second most common histological subtype of PTC, accounting for 9~20% of patients with PTC [2–4]. This variant has the typical nuclear features of PTC with a predominantly follicular histological growth pattern [5]. FVPTCs are classified into encapsulated and infiltrative forms [6]. Studies have shown that encapsulated FVPTC has lower risks of recurrence and metastasis than the infiltrative form [7–9]. Recently, it has been reported that encapsulated FVPTCs without capsular or vascular invasion have excellent clinical outcomes with a very low risk of recurrence [8–12]. Based on these findings, a nomenclature revision from

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"non-invasive encapsulated FVPTC" to "non-invasive follicular thyroid neoplasm with papillary-like nuclear feature (NIFTP)" was recently proposed [13]. This change in nomenclature might promote the conservative management of NIFTPs.

It has been shown that encapsulated and infiltrative FVPTCs have different molecular profiles [8, 14–16]. Encapsulated FVPTCs harbored a high frequency of *RAS* mutations (30~40%) but lacked *BRAF* mutations [8, 14, 16]. In contrast, infiltrative tumors had a higher rate of *BRAF* mutation and a lower rate of *RAS* mutation [8, 15]. Recent studies have compared the mutational statuses of *BRAF* and *RAS* between non-invasive and invasive encapsulated FVPTCs [15, 17]. NIFTP is characterized by the absence of the $BRAF^{V600E}$ mutation [17], whereas ~30% of invasive encapsulated FVPTCS were reported to harbor $BRAF^{V600E}$ [17, 18]. However, a recent study from Korea reported that NIFTP harbored $BRAF^{V600E}$ at a frequency of 28.6% [15]; therefore, controversy remains and a comprehensive analysis is required.

Given the heterogenous features of FVPTCs and the increased recognition of NIFTP, we evaluated each subtype of FVPTC that was histologically diagnosed at our institution and aimed to compare the genetic and clinicopathologic characteristics of the subtypes.

Methods

Subjects

This study evaluated patients who underwent thyroidectomy at Myongji-hospital (Goyang, Korea) between January 2007 and June 2017. Among 296 patients diagnosed with PTC, 34 were diagnosed with FVPTC based on the surgical pathology reports. Among them, 29 cases with available tissue blocks for molecular analysis were selected. The electronic medical records of each patient were retrospectively reviewed. The study protocol was approved by the institutional review board of Myongji-hospital, Goyang, Korea (IRB no. MJH-17-013).

Pathological Analysis

Preoperative fine needle aspiration cytology (FNAC) results were categorized using the Bethesda system [19]. FVPTCs were defined as follicular lesions that are almost entirely composed of follicles and have the nuclear features of PTC. All histology slides were examined independently by two experienced pathologists with a special interest in thyroid pathology (J.J. Lee and Y.Y. Jung). FVPTCs were classified as encapsulated or infiltrative FVPTC, as previously described [8] (Fig. 1).

Briefly, tumors with a complete fibrous capsule delineating the tumor from the non-tumor thyroid parenchyma were classified as encapsulated. In each case of encapsulated FVPTC, a complete analysis of the tumor capsule interface was performed. For this purpose, thyroid specimens were entirely sectioned to obtain 3-mm-thick tissue slices from the capsular interface. These were then routinely processed to obtain a formalin-fixed paraffin-embedded tissue block. Finally, five serial sections were cut and stained with hematoxylin and eosin. Encapsulated FVPTCs were categorized into NIFTP and invasive encapsulated FVPTC. Diagnostic criteria for the NIFTP included follicular growth pattern with less than 1% papillae, encapsulation with clear demarcation from adjacent normal thyroid parenchyma, presence of nuclear features of PTC according to the consensus criteria by Nikiforov et al [13]. Exclusion findings were as follows: 1) psammoma bodies; 2) more than 30% solid, trabecular, or insular growth pattern; 3) tumor necrosis; and 4) high mitotic activity ($\geq$ 3 mitoses per 10 high-power fields. These tumors showed no capsular or vascular invasion [13]. If any capsular or vascular invasion in the tumor capsule was observed, the tumor was classified as invasive FVPTC. Infiltrative FVPTCs were defined as tumors with invasive tongues infiltrating into the non-neoplastic thyroid parenchyma [7].

Next-Generation Sequencing

DNA Extraction, Library Preparation and Sequencing

Twenty-nine tissue specimens were macrodissected from 8 to 10-μm-thick unstained archived formalin-fixed paraffin-embedded sections. Tumor presence was verified by hematoxylin and eosin staining. Areas containing viable tumors were marked on the slides. In comparison with non-tumor tissue components, the dissected areas contained at least 80% tumor cells. Paraffin was removed by xylene treatment, and DNA was extracted using a QIAamp® DNA FFPE tissue kit (Qiagen, Hilden, Germany). DNA concentration and purity were checked using a Nanodrop 8000 UV-Vis spectrometer (Thermo Scientific, Waltham, MA, USA) and Qubit 2.0 Fluorometer (Life Technologies, Grand Island, NY, USA). The degree of DNA degradation was measured using a 200 TapeStation Instrument (Agilent Technologies, Santa Clara, CA, USA). Genomic DNA was sheared using a Covaris S220 (Covaris, Woburn, MA). Target capture was performed using the SureSelect XT Reagent Kit, HSQ (Agilent Technologies) and a paired-end sequencing library was constructed with a barcode. After checking for library quality, sequencing was performed on a HiSeq 2500 with 100-bp reads (Illumina, San Diego, CA, USA). A targeted panel was used to capture 83 cancer-related genes, including all coding exons (Supplemental Table 1).

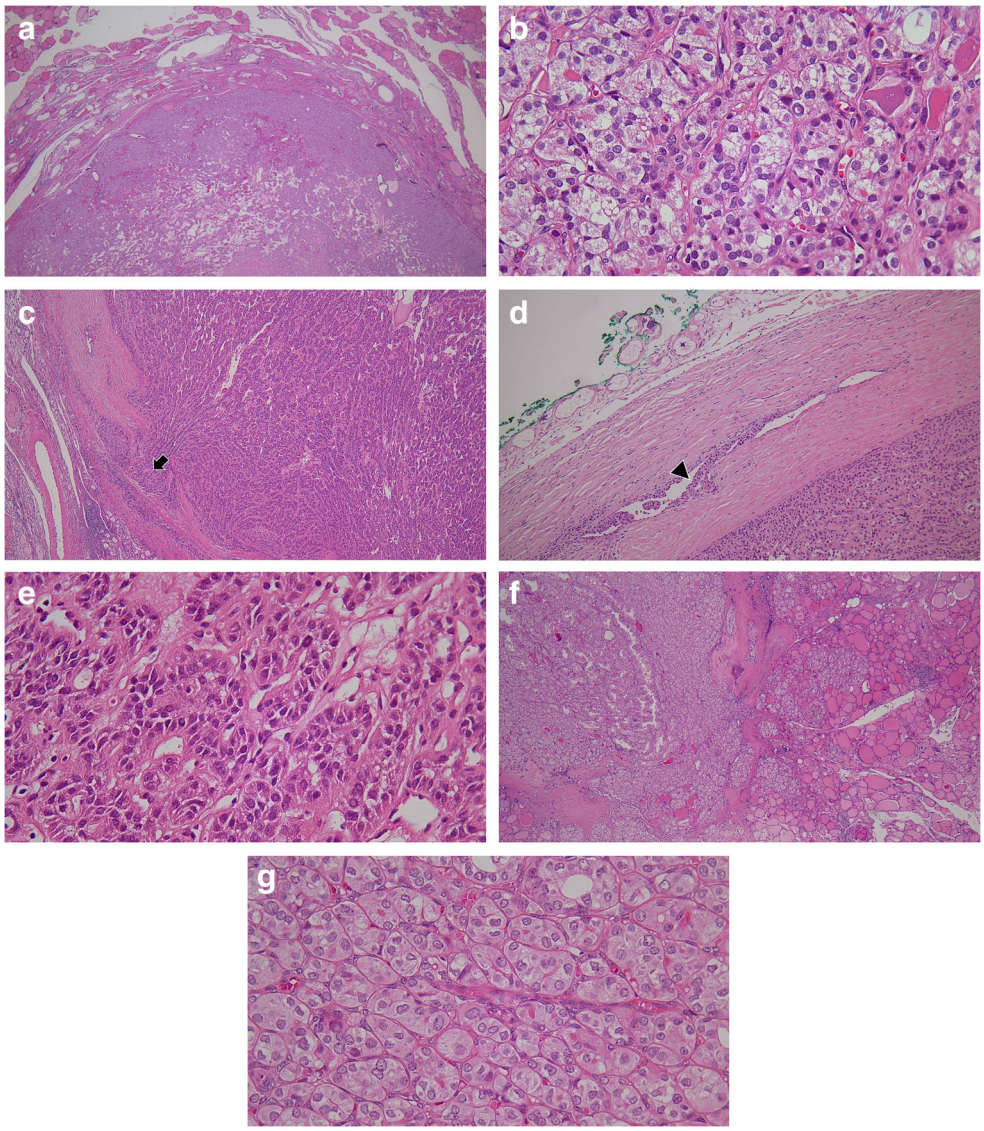

Fig. 1 Microscopic features of the follicular variant papillary thyroid carcinoma (FVPTC). a-b, non-invasive encapsulated type, **c-e**, invasive encapsulated type and **f, g** infiltrative type. (**a**) A follicular proliferative tumor is circumscribed by a fibrous capsule without capsular or vascular invasion (H&E, ×12.5). **c** A follicular proliferative tumor invades tumor capsule (arrow, H&E, ×12.5). **d** The tumor also shows vascular invasion (arrow head, H&E, ×100). **f** A follicular proliferative tumor has an infiltrative tumor border with dense fibrosis (H&E, ×40). **b**, **e** and **g** All FVPTCs show nuclear enlargement, irregular nuclear contours, pale chromatin, nuclear grooves and pseudoinclusions (H&E, ×400)

Variant Detection and Data Processing

The raw sequence reads were processed and aligned to the hg19 human reference sequence with the Burrows–Wheeler Aligner [20]. Duplicate reads were removed with Picard, and local alignment optimization and base recalibration were performed with the Genome Analysis Tool Kit (GATK) [21]. We recalibrated base quality scores using GATK based on known single-nucleotide polymorphisms and insertion and deletions from dbSNP138. To increase sensitivity, we used two published methods for single nucleotide variants detection, MuTect13 (v1.1.4) and LoFreq14 (v0.6.1), with default parameters. The union of the variants identified by the two callers (with the high confidence set for MuTect) was used as the candidate set of variants. Small insertion and deletions were identified by Pindel34 (v0.2.4) with its default setting. We applied several filtering steps to exclude these putative germline variants: (i) variants with very high VAF (≥97%), except for the hotspot mutations; (ii) variants with population allele frequency > 3% in the >400 normal samples in our database; and (iii) other frequently detected variants that are likely to be alignment artifacts or are in hard-to-sequence regions, as curated by manual review. The single nucleotide variants and insertion and deletions were annotated by ANNOVAR [22]. Several databases were additionally used for annotation such as SnpEff [23], ClinVar and COSMIC databases to analyze their clinical significance [24]. Single-nucleotide variants with a variant allele frequency of 5% or greater and an insertion/deletion frequency of 10% or greater were finally selected [24, 25]. All retained alterations were then classified as pathogenic variants or variants of unknown significance according to the following criteria. Pathogenic variants were defined as known pathogenic missense, frameshift, nonsense, or splice-site mutations.

Variants of unknown significance were defined as missense, nonsense and frameshift mutations with unknown functional effects or losses of uncertain function.

Statistical Analysis

A Fisher's exact test was used to assess the relationships between categorical variables, and Student's *t* test was used for continuous variables. A *P* value of <0.05 was considered to be statistically significant. Statistical analyses were performed using SPSS version 20 (IBM, Armonk, NY, USA).

Results

Clinical Characteristics and Histopathological Features

The baseline clinical data and pathological characteristics according to the three subgroups of the FVPTCs are described in Table 1. Considering the preoperative FNAC results, the follicular neoplasm or suspicious for follicular neoplasm category was the most common preoperative FNAC diagnosis among the NIFTPs, and there was no significant difference between NIFTPs and invasive encapsulated FVPTCs.

On the pathological reports, there were no significant differences in tumor size, tumor encapsulation, and gross extrathyroidal extension between NIFTPs and invasive encapsulated FVPTCs. Cervical LN metastasis was not found in NIFTP. Initial American Thyroid Association (ATA) risk classification was comparable between two groups.

Between encapsulated cases and infiltrative cases, infiltrative FVPTCs had a higher rate of malignancy based on diagnosis by FNAC (50% vs. 9.5%, $P = 0.033$) and initial total thyroidectomy tended to be performed more frequently in infiltrative FVPTCs than in encapsulated FVPTCs ($P = 0.075$). Patients with infiltrative FVPTCs were older (55.1 ± 6.2 vs. 48.4 ± 10.2 years, $P = 0.044$) and had small tumors than those with encapsulated FVPTCs (1.0 ± 0.4 vs. 1.7 ± 1.2 cm, $P = 0.013$). Gross extrathyroidal extension was found more frequently in infiltrative cases ($P = 0.018$). Patients with infiltrative FVPTCs presented with advanced ATA risk as compared with patients with encapsulated FVPTC.

The overall clinical outcomes of FVPTCs were favorable during a median follow-up period of 71 months, and only one patient with an invasive encapsulated FVPTC showed a small suspicious LN after the initial surgery.

Molecular Analysis by Next-Generation Sequencing

The detected molecular alterations of each patient with FVPTC are presented in Table 2. Variants with low allelic fraction and variants of unknown significance are presented in supplemental Table 2. Genetic mutations were found in 5 of 10 NIFTPs, 7 of 11 invasive encapsulated FVPTCs, and 6 of 8 infiltrative FVPTCs. The most common genetic alterations identified in NIFTPs were mutations in *RAS* ($n = 5$, 50%) *NF1* mutations was found in two NIFTPs and these were coexistent with *RAS* mutation. *BRAF* mutation was not found in the NIFTPs.

Among the 11 cases with invasive encapsulated FVPTCs, the most common mutations were in *RAS* ($n = 4$, 36.4%) and *NF1* ($n = 4$, 36.4%). $BRAF^{V600E}$ mutation was found in one case. The *NF1* mutations had various amino acid changes.

Six of eight infiltrative FVPTCs harbored at least one genetic alteration. The most frequently identified alteration was a *BRAF* mutation ($n = 4$, 50%). An *NRAS* mutation in codon 61 was detected in one infiltrative FVPTC. *CTNNB1* mutation was detected in one case.

Prevalence of *BRAF* and *RAS* Mutations in each FVPTC Subtype

We compared the frequency of *BRAF* and *RAS* mutations among the subtypes of FVPTC (Table 3). There were four *BRAF* mutations (50%) in infiltrative FVPTCs and one *BRAF* mutation in invasive encapsulated FVPTC (9.1%), while none of NIFTPs had a *BRAF* mutation. Among the five *BRAF* mutations, four were $BRAF^{V600E}$ and one was $BRAF^{V600\_K601>E}$. *BRAF* mutations were highly prevalent in infiltrative FVPTCs as compared with the encapsulated FVPTCs (62.5% vs. 4.8%, $P = 0.003$). Additionally, $BRAF^{V600E}$ was more frequently detected in infiltrative FVPTCs than in encapsulated FVPTCs (37.5% vs. 4.8%, $P = 0.022$).

Among the 21 cases of encapsulated FVPTCs, $BRAF^{V600E}$ was found in one case (Fig. 2a). Microscopic examination showed a focus of capsular invasion (Fig. 2b). The tumor had abortive papillae less than 1% (Fig. 2c).

The $BRAF^{V600\_K601>E}$ mutation was detected in one infiltrative FVPTC by next-generation sequencing (Fig. 3a). Microscopic examination revealed an infiltrative tumor border with foci of capsular invasion without vascular invasion (Fig. 3b).

RAS mutations were identified in 5 NIFTPs (50%), 4 invasive encapsulated FVPTCs (36.4%), and one infiltrative FVPTC (12.5%). Among the encapsulated FVPTCs, an *NRAS* mutation in codon 61 was the most common mutation (6 of 9 *RAS*-positive tumors) followed by a *KRAS* mutation in codon 61 (3 of 9 *RAS*-positive tumors). Encapsulated FVPTCs tended to have a higher frequency of *RAS* mutations than infiltrative FVPTCs, but this trend was not statistically significant ($P = 0.081$). There was no significant difference in the frequency of *RAS* or *NRAS* mutations between NIFTPs and invasive encapsulated FVPTCs.

Table 1 Clinicopathologic characteristics of patients according to the subtype of FVPTC

	Encapsulated FVPTC		Infiltrative FVPTC	*P* value	
Characteristics	Non-invasive	Invasive		Non-invasive vs. invasive	Encapsulated vs. infiltrative
Age (years)	50.5 ± 9.7	46.6 ± 10.8	55.1 ± 6.2	0.390	0.044
Male sex, *n* (%)	3 (30%)	3 (27.2%)	0 (0%)	0.89	0.633
Type of surgery					
Lobectomy	5 (50%)	3 (27.2%)	2 (25%)	0.284	0.507
Lobectomy with completion	1 (10%)	4 (36.4%)	0	0.157	0.129
Total thyroidectomy	4 (50%)	4 (36.4%)	6 (75%)	0.864	0.075
Radioactive iodine					
No	7 (70%)	6 (54.5%)	4 (50%)	0.659	0.683
Yes	3 (30%)	5 (45.5%)	4 (50%)		
Preoperative cytology					
Benign	2 (20%)	2 (18%)	0	0.669	0.552
AUS	3 (30%)	1 (9%)	2 (25%)	0.311	0.543
FN/SFN	4 (40%)	3 (27%)	2 (25%)	0.659	0.517
Suspicious for malignancy	0	3 (27%)	0	0.074	0.54
Malignant	1 (10%)	1 (9%)	4 (50%)	0.738	0.033
Tumor size	2.0 ± 1.3	1.5 ± 0.8	1.0 ± 0.4	0.291	0.013
Tumor capsule					
Totally encapsulated	5 (50%)	5 (45.5%)	0	0.99	0.016
Partially encapsulated/well circumscribed	5 (50%)	6 (54.5%)	0	0.99	0.009
Infiltrative	0	0	8 (100%)	0.99	<0.001
Growth pattern					
Microfollicular	8 (80%)	9 (81.8%)	5 (62.5%)	0.916	0.282
Macrofollicular	2 (20%)	2 (18.2%)	3 (37.5%)		
Lymph node metastasis					
Nx	2 (20%)	4 (36.4%)	1 (12.5%)	0.407	0.366
N0	8 (80%)	6 (54.5%)	5 (62.5%)	0.217	0.833
N1a	0	1 (9.1%)	2 (25%)	0.329	0.11
Vascular invasion					
Absent	10 (100%)	10 (90.9%)	8 (100%)	0.99	0.53
Present	0	1 (9.1%)	0		
Gross extrathyroidal extension					
Absent	10 (100%)	11 (100%)	6 (75%)	0.99	0.018
Present	0	0	2 (25%)		
Initial ATA risk classification					
Low	9 (90%)	10 (90.9%)	5 (62.5%)		
Intermediate	1 (10%)	1 (9.1%)	1 (12.5%)		
High	0	0	2 (25%)	0.99	0.018
Structural recurrent or persistent disease	0	1	0	0.329	0.53
Follow up duration (months)	66 (10–84)	78 (10–97)	49 (26–99)	0.353	0.98

FVPTC, follicular variant papillary thyroid cancer; AUS, atypia of undetermined significance; FN, follicular neoplasm; SFN, suspicious for follicular neoplasm; ATA, American Thyroid Association

Discussion

In this study, we evaluated the molecular and clinicopathologic features of FVPTC. NIFTP had molecular and pathological profiles that were similar to those of invasive encapsulated FVPTC, but distinct from those of infiltrative FVPTC. There was no *BRAF* mutation in our series of NIFTPs, while *RAS* mutations were highly prevalent in both NIFTPs and invasive encapsulated FVPTCs. These findings suggest that NIFTP should be considered as an

Table 2 Mutational profile of each patient with FVPTC

Patient No.	Pathologic subtype	TNM stage*	Gene	Allelic fraction	Amino Acid Change
1	NIFTP	T1bNxM0	*NRAS*	14%	Q61R
			NF1	7%	Q589*
2	NIFTP	T1aN0M0	*NRAS*	31%	Q61K
3	NIFTP	T1bN0M0	*NRAS*	8%	Q61R
4	NIFTP	T2N0M0	*NRAS*	19%	Q61R
5	NIFTP	T1bN0M0	*KRAS*	6%	Q61R
			NF1	8%	R659Q
6	NIFTP	T3aN0M0	none	–	–
7	NIFTP	T1bN0M0	none	–	–
8	NIFTP	T1aNxM0	none	–	–
9	NIFTP	T2N0M0	none	–	–
10	NIFTP	T1aN0M0	none	–	–
11	Invasive EFVTPC	T2N0M0	*NRAS*	17%	Q61R
12	Invasive EFVPTC	T1bN0M0	*NRAS*	5%	Q61R
			NF1	8%	F1287 L
13	Invasive EFVPTC	T2NxM0	*KRAS*	26%	Q61R
			NF1	10%	T467I
14	Invasive EFVPTC	T2N0M0	*KRAS*	31%	Q61R
15	Invasive EFVPTC	T1aN1aM0	*BRAF*	9%	V600E
16	Invasive EFVPTC	T1aN1aM0	*NF1*	8%	T467I
17	Invasive EFVPTC	T1aN0M0	*NF1*	8%	M1022 T
18	Invasive EFVPTC	T1bN0M0	none	–	–
19	Invasive EFVPTC	T1bNxM0	none	–	–
20	Invasive EFVPTC	T1aNxM0	none	–	–
21	Invasive EFVPTC	T1bNxM0	none	–	–
22	Infiltrative FVPTC	T1aNxM0	*CNNNB1*	7%	R225C
23	Infiltrative FVPTC	T4aN1aM0	*BRAF*	11%	V600E
24	Infiltrative FVPTC	T1bN1aM0	*BRAF*	19%	V600E
25	Infiltrative FVPTC	T1aN0M0	*BRAF*	10%	V600_K601 > E
26	Infiltrative FVPTC	T1aN0M0	*BRAF*	5%	V600E
27	Infiltrative FVPTC	T1bN0M0	*NRAS*	13%	Q61R
28	Infiltrative FVPTC	T1aN0M0	none	–	–
29	Infiltrative FVPTC	T4aN0M0	none	–	–

*T and N stages were classified using the 8th edition of the TNM staging system

NIFTP, noninvasive follicular thyroid neoplasm with papillary-like nuclear features; invasive EFVPTC, invasive encapsulated follicular variant papillary thyroid carcinoma; infiltrative FVPTC, infiltrative follicular variant papillary thyroid carcinoma

early stage of invasive encapsulated FVPTC, particularly in tumors driven by *RAS* mutations.

In the present study, the $BRAF^{V600E}$ mutation was not detected in NIFTP, while it was identified in 9.1% of invasive encapsulated FVPTCs and 37.5% of infiltrative FVPTCs. The absence of $BRAF^{V600E}$ has been regarded as a characteristic features of NIFTP [8, 17], while the *BRAF* mutation was previously detected in 0–30% of invasive encapsulated FVPTCs [17, 18, 26] and infiltrative FVPTCs [8]. However, two recent studies from Korea reported that the *BRAF* mutation was present in 8% [27] and 28.6% of NIFTPs [15], respectively. Recently, it has been suggested that more strict histologic criteria are needed to establish the diagnosis of NIFTP. Studies suggested that the absence of papillary structures should be a diagnostic criterion of NIFTP instead of a frequency of papillae <1% [28, 29]. Cho et al. provided evidence that all $BRAF^{V600E}$-harboring encapsulated FVPTCs could be reclassified as classic PTCs with predominant follicular growth using rigid histologic criteria [28]. When we reviewed a slide of one invasive encapsulated FVPTC harboring $BRAF^{V600E}$ in our study, the tumors had abortive papillae at a frequency of less than 1%. If we applied the absence of papillary structures as a criteria for encapsulated FVPTC, then we would conclude that $BRAF^{V600E}$ was not found in encapsulated FVPTCs.

Table 3 Prevalence of *BRAF* and *RAS* mutations according to the subtype of FVPTC

	Encapsulated FVPTC		Infiltrative FVPTC	*P* value	
	Non-invasive	Invasive		Non-invasive vs. invasive	Encapsulated vs. infiltrative
BRAF mutation	0	1 (9.1%)	4 (50%)	0.329	0.003
BRAF p.V600E	0	1 (100%)	3 (60%)	0.329	0.022
BRAF p.V600_K601 > E	0	0	1 (20%)	–	0.099
RAS mutation	5 (50%)	4 (36.4%)	1 (12.5%)	0.528	0.081
NRAS p.Q61R	3 (60%)	2 (50%)	1 (100%)	0.525	0.502
NRAS p.Q61K	1 (20%)	0	0	0.283	0.530
KRAS p.Q61R	1 (20%)	2 (50%)	0	0.593	0.259

FVPTC, follicular variant papillary thyroid carcinoma

One patient with infiltrative FVPTC in our study had a $BRAF^{V600\_K601>E}$ mutation. This *BRAF* mutation results from a triplet deletion of TGA (coding nucleotides 1799–1801) in exon 15 of *BRAF* [27]. The in-frame deletion of TGA (c.1799_1801delTGA) leads to a deletion of lysine at codon 601 and a valine-to-glutamate substitution at codon 600 in the resultant BRAF protein (p.V600_K601 > E). This *BRAF* mutation was first described in a case of solid variant PTC [30], but has also been reported in some cases of encapsulated FVPTC [31] and classic PTC [32]. Given the rarity of *BRAF* deletions, it is unclear whether the mutation is associated with a specific histological type of PTC.

In our series, genetic alterations other than *BRAF* or *RAS* were also detected in patients with FVPTCs using next-generation sequencing. *NF1* mutations were frequently detected in encapsulated FVPTC (two cases of 10 NIFTPs and four cases of 11 encapsulated FVPTCs) but not found in infiltrative FVTPCs. Among the six encapsulated FVPTCs harboring *NF1* mutations, four had concurrent *RAS* mutations. Recent studies have identified *NF1* mutations in follicular thyroid tumors, including a

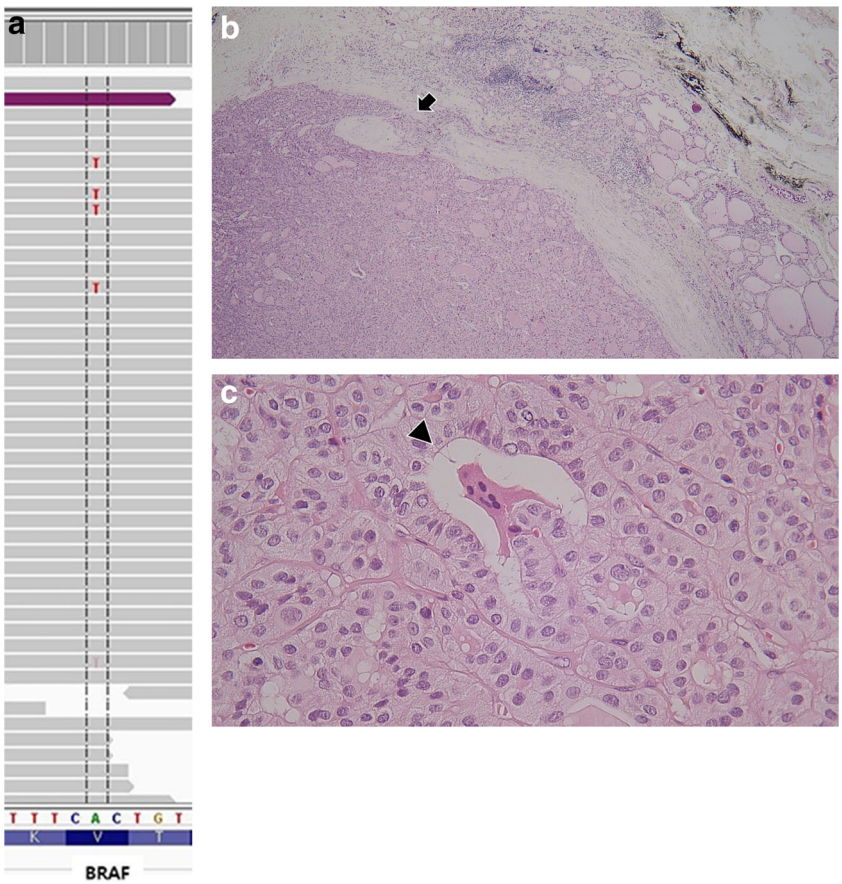

Fig. 2 A case of invasive encapsulated FVPTCs harboring $BRAF^{V600E}$ mutation. a $BRAF^{V600E}$ mutation is detected by next generation sequencing (**b**) Microscopic examination reveals a focus of capsular invasion (arrow, H&E, ×40). **c** There is a papilla (arrow head), but it is not a true papilla

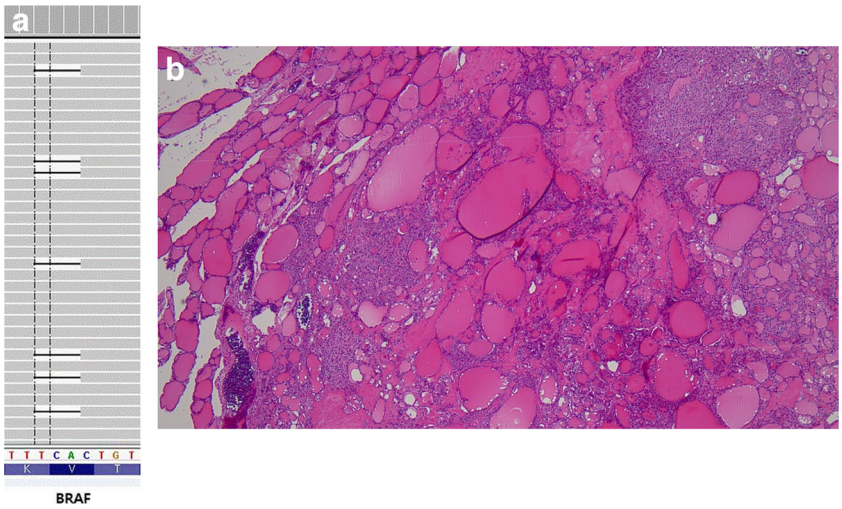

Fig. 3 A case of infiltrative FVPTCs harboring *BRAF*$^{V600\_K601>E}$ mutation. a *BRAF*$^{V600\_K601>E}$ mutation is detected by next generation sequencing. **b** Microscopic examination reveals an infiltrative tumor border (H&E, ×40)

small proportion of follicular thyroid adenomas [33] and follicular thyroid carcinomas [34]. The *NF1* mutations detected in our series exhibited nucleotide changes at various positions and had an allelic frequency of 6–10%. This may suggest that *NF1* mutations are not the sole driving mutations for encapsulated FVPTCs. Among the 29 FVPTCs in our study, *CTNNB1* mutations were found in one infiltrative FVPTC. In contrast to our study, previous studies have suggested that *CTNNB1* mutations contribute to the tumorigenesis of aggressive follicular cell-derived thyroid cancer [35, 36]. The *NF1* and *CTNNB1* mutations showed a relatively lower allelic fraction compared to known driver *BRAF* and *RAS* mutation. Therefore, these mutation might be subclonal events in FVPTC. Further large-scale comprehensive studies are needed to confirm these findings.

In our study, some FVPTCs had driver mutations at low allelic fraction with a frequency of 5~10%. This finding suggests that intra-tumor clonal heterogeneity may exist during tumor evolution. Guerra et al. reported that 66% of the PTCs harbored *BRAF* mutations with an allelic fraction of less than 25% which suggest subclonal *BRAF* occurrence [37]. In addition, the presence of wild type stromal cells may lead to a partial underestimation of mutated allele fraction in some of the tumors, despite we selected tumor tissue carefully to minimize this bias.

It has been reported that the pre-operative cytologic diagnosis of FVPTCs with FNA is difficult, particularly for the encapsulated type, because these tumors may not show the typical cytological features of classic PTC [38]. Most NIFTPs have nuclear features and cytological classifications that overlap with those of non-NIFTP FVPTCs [15, 18]. Additionally, indeterminate cytological features paired with a *RAS* mutation on molecular testing may increase the suspicion for FVPTC [31] or NIFTP [39]. Paulson et al. found that NIFTPs accounted for 63% of *RAS* mutant tumors with a prior indeterminate FNA diagnosis [39]. In our series, a significant proportion of NIFTPs and invasive encapsulated FVPTCs were classified as indeterminate categories and harbored *RAS* mutations [27]. Taken together, it seems to be impossible to distinguish between NIFTP and invasive encapsulated FVPTC preoperatively based on cytology and molecular testing.

In the present study, patients with FVPTCs had favorable clinical outcomes, similar to the previous study [27, 40]. Only one patient with invasive encapsulated FVPTC showed structural persistent disease after initial treatment. LN metastasis, which is one of the aggressive features of PTC, was not found in NIFTPs. The initial ATA risk of recurrence was comparable between NIFTP and invasive encapsulated FVPTC; however, the high-risk category was present only in infiltrative FVPTC. Therefore, for most patients with either NIFTP or invasive encapsulated FVPTC, lobectomy without complete thyroidectomy as an initial treatment would be sufficient, as previously suggested [41].

In conclusion, NIFTP has molecular profiles and clinicopathologic characteristics similar to those of invasive encapsulated FVPTCs but different from those of infiltrative FVPTCs. Because of the similar frequencies of RAS mutation and cytologic classifications in both NIFTP and invasive encapsulated FVPTC, it seems to be difficult to distinguish NIFTP from invasive encapsulated FVPTC preoperatively. NIFTP has favorable pathologic characteristics with a high frequency of *RAS* mutations.

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Compliance with Ethical Standards

Conflict of Interest All authors have no conflict of interest.

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