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Immunohistochemical and Molecular Characteristics of Follicular Patterned Thyroid Nodules with Incomplete Nuclear Features of Papillary Thyroid Carcinoma

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Background : Follicular patterned thyroid nodules with incomplete nuclear features of papillary thyroid carcinoma (FTN-INPTCs) are difficult to diagnose, and their biological behavior and association with follicular variants of PTC (FVPTCs) have not yet been established. The aim of this study is to determine immunohistochemical and molecular characteristics of FTN-INPTCs. **Methods :** We investigated immunohistochemical features (galectin-3, HBME-1, CK19, fibronectin-1, CITED1), *BRAF* V600E mutation and *RASSF1A* promoter methylation status in 30 FTN-INPTC cases, along with 26 FVPTCs, 21 follicular adenomas (FAs) and 14 nodular hyperplasias (NHs). **Results :** Expression of galectin-3, HBME-1, CK19 and CITED1 was significantly higher in FTN-INPTCs than in FAs or NHs, but expression of galectin-3, CK19 and fibronectin-1 was lower in FTN-INPTCs than in FVPTCs. The *BRAF* V600E mutation was not detected in the benign nodules or FTN-INPTCs, whereas 57% of FVPTCs had the mutation. *RASSF1A* promoter methylation was higher in FTN-INPTCs than in benign nodules but there was no difference between FTN-INPTCs and FVPTCs. **Conclusions :** Our results represent the borderline immunohistochemical and molecular characteristics of FTN-INPTC. We conclude that FTN-INPTC is an intermediate lesion between a benign nodule and a FVPTC, and that it is pathogenetically related to FVPTC.

Key Words : Thyroid gland; Thyroid neoplasms; Carcinoma, Papillary

Follicular variant of papillary thyroid carcinoma (FVPTC) is a special subtype of papillary thyroid carcinoma (PTC). It resembles follicular neoplasm and is almost exclusively composed of follicles without papillary structures. Thus, its diagnosis is based on the characteristic nuclear features of PTC, including enlargement, ground glass appearance, grooves, and pseudoinclusions.[1] However, atypical cells whose nuclei have a clear chromatin pattern, membrane irregularity, grooves, or even pseudo-inclusions are occasionally observed in benign thyroid nodules such as nodular hyperplasia (NH) or follicular adenoma (FA). These features resemble the characteristic nuclei of papillary carcinoma but the degree of nuclear atypia is incomplete for the definite diagnosis of FVPTC. The diagnostic difficulty of this "follicular patterned thyroid nodule with incomplete nuclear features of papillary thyroid carcinoma (FTN-INPTC)" has been mentioned previously.[2] The use of the diagnostic term "well differentiated tumor of uncertain malignant potential (WDTUMP)" was suggested by the Chernobyl Pathologists Group to designate an encapsulated and non-invasive tumor with equivocal or questionable nuclear features of PTC, emphasizing the potential for malignant transformation of this lesion.[3,4]

PTCs have critical genetic alterations associated with their de-

velopment, such as *RET/PTC* or *NTRK1* rearrangements, and *BRAF* V600E or *RAS* mutations.[5] The *BRAF* V600E mutation is found in 29-83% of PTCs,[6,7] making it the most common genetic event in PTC. In addition, a few epigenetic changes have been identified in PTCs, including silencing of *RASSF1A* (RAS association domain family protein 1A). *RASSF1A* is a tumor suppressor gene inhibiting cell cycle progression,[5] which is regulated by promoter hypermethylation. It is silenced in 20-62% of PTCs and it has been reported that its silencing and the *BRAF* mutation are mutually exclusive.[8,9] Interestingly, *RASSF1A* promoter methylation is also found in follicular adenoma, suggesting that epigenetic inactivation of *RASSF1A* is an early step in thyroid tumorigenesis.

In this study, we investigated immunohistochemically the expression of several markers in FTN-INPTCs. These included galectin-3,[10,11] HBME-1,[4,12,13] cytokeratin 19 (CK19),[13,14] fibronectin-1,[15] and CITED1,[15] which have been reported to be markers of PTC. We also examined *BRAF* V600E mutation and *RASSF1A* promoter methylation in FTN-INPTCs. By comparing these results with those of FVPTCs, FAs and NHs, we tried to determine the histological features, immunohistochemical and molecular characteristics of this atypical follicular lesion.

MATERIALS AND METHODS

Tissue specimens and histopathologic evaluation

Ninety-one cases of surgically resected thyroid lesions including 26 FVPTCs, 30 FTN-INPTCs, 21 FAs and 14 NHs were collected from files in the Department of Pathology, Seoul National University Bundang Hospital from 2004 to 2007. Twenty-two of the patients were male (24%) and 69 female (76%) with a mean age of 49 ± 12.6 years (mean ± standard deviation; range, 17-80 years).

Pathologic information was obtained from hematoxylin and eosin (H-E)-stained sections. FTN-INPTC was defined as follows: a follicle-forming nodule whose nuclei have similar but incomplete and not identical features to PTC, such as chromatin clearing, grooves, membrane irregularity, overlapping, and pseudoinclusions. The following clinicopathologic variables were recorded in FTN-INPTCs: distribution of nuclear atypia (diffuse or focal), overall features of FTN-INPTC i.e. lesion (FA or NH) in which incomplete nuclear features of PTC are observed, and presence of concurrent PTC in the surrounding thyroid parenchyma. FVPTC cases were defined as follicle-forming tumors having the characteristic nuclear features of PTC without any visible papillary architecture, irrespective of encapsulation. All cases were independently reviewed and confirmed by two endocrine pathologists.

Immunohistochemistry and interpretation

Representative paraffin blocks were selected for immunohistochemistry. Four micron-thick sections were deparaffinized, rehydrated in a series of alcohols, and processed with a DAKO-Envision detection kit (DakoCytomation, Carpinteria, CA, USA). Antigen was retrieved in a microwave oven for 15 min in 10 mmol/L citrate buffer pH 6.0. Endogenous peroxidase activity was blocked with a 3% H_2O_2-methanol solution, and the slides were incubated in 10% normal goat serum for 10 min to prevent nonspecific staining. They were then incubated for 1 h at room temperature with an appropriately diluted primary antibody. The following antibodies were used: galectin-3 (9C4; 1:600; Novocastra, UK), HBME-1 (HBME-1; 1:100; DakoCytomation), CK19 (RCK108; 1:150; DakoCytomation), fibronectin-1 (polyclonal; 1:2,000; DakoCytomation), CITED1 (polyclonal; 1:50; Abcam, UK). Thereafter, the sections were incubated with horseradish peroxidase-labeled polymer conjugated with secondary antibodies for 30 min. Diaminobenzidine was used as a chromogen, and the sections were counterstained with Mayer's hematoxylin. As a negative control, non-immune serum was substituted for the primary antibody.

Galectin-3 and CITED1 expression was both nuclear and/or cytoplasmic. HBME-1 was expressed in the cytoplasm and cell membrane with occasional luminal accentuation. CK19 expression was cytoplasmic with membranous accentuation. Fibronectin-1 was expressed in the cytoplasm.

The expression of markers was classified as follows: negative, no staining or staining in less than 10% of the cells of a lesion; positive, staining in more than 10% of the cells. Staining of 10-50% of the cells was defined as focal positive, and staining of more than 50% was defined as diffuse positive.

Detection of the BRAF mutation

Genomic DNA was extracted from all cases and sequencing was possible in 77 cases, including 32 benign nodules, 24 FTN-INPTCs, and 21 FVPTCs. Briefly, representative areas were marked with 4 micron-thick H-E stained sections as guides. Then, the marked areas were matched with de-waxed but unstained 10 micron-thick sections. The tumor areas were dissected from

the unstained slides and transferred into Eppendorf tubes. After dissection, the blocks were cut into 4 micron sections for H-E staining to confirm tumor continuity. All the samples were then digested with proteinase K for more than 24 h at 56˚C, and DNA was isolated from the digested tissue using a General Biosystems Tissue SV mini kit (General Biosystems, Seoul, Korea). We amplified *BRAF* exon 15 by the polymerase chain reaction (PCR) using primers: forward, 5′-GCTTGCTCTGATAGGAAAATGAG-3′, reverse, 5′-GATACTCAGCAGCATCTCAGG-3′. PCR conditions were: 95˚C for 5 min, 40 cycles at 94˚C for 20 s, 56˚C for 20 s, 72˚C for 20 s, and 72˚C for 10 min. We sequenced the purified PCR products in an MJ Research PTC-225 Peltier Thermal Cycler using ABI PRISM® BigDye™ Terminator Cycle Sequencing Kits and AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems, Foster city, CA, USA). Single-pass sequencing was performed on each template using the forward primer. The fluorescence-labeled fragments obtained were purified from unincorporated terminators by ethanol precipitation, resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems).

Real-time quantitative methylation-specific PCR (MSP) for RASSF1A

The genomic DNA was converted with sodium bisulfite as described previously,[16] and the DNA was purified using a QIAamp viral RNA mini kit (Qiagen, Valencia, CA, USA). For real-time quantitative MSP, we used the MethyLight assay.[17] The PCR amplification was performed using TaqMan® 1,000 RXN Gold supplied with the Buffer A Pack (Applied Biosystems), in a total volume of 30 μL containing 10 μL DNA, 3.5 mmol/L $MgCl_2$, 3.0 μL 10 × buffer, 200 μmol/L each of dNTP, 0.3 nmol/L each of forward and reverse primers, 0.3 nmol/L TaqMan probe, and 0.5 μL AmpliTaq Gold DNA polymerase (5 U/μL). Real-time PCR reactions were followed with an ABI Prism 7,700 Sequence Detection System (Applied Biosystems). The primers and probe designed to specifically amplify bisulfite-converted DNA in the promoter region of the methylated version of *RASSF1A* gene were used: forward 5′-GCG TTG AAG TCG GGG TTC-3′; reverse 5′-CCC GTA CTT CGC TAA CTT TAA ACG-3′; probe 6FAM-5′-ACA AAC GCG AAC CGA ACG AAA CCA-3′-TAMRA, and primers and probe for the ALU-based MethyLight control reaction were also used, to normalize data.[17] The percentage of fully methylated DNA at a *RASSF1A* gene was calculated by dividing the *RASSF1A:ALU* ratio of a sample by the *RASSF1A:ALU* ratio of SssI-treated sperm DNA and multiplying by 100.[18] The calculated values were expressed as percent of methylated reference (PMR). For the analysis, lesions were considered to be methylated when their PMR >4, and unmethylated when their PMR $\leq$4.

Statistical analysis

Statistical analysis was performed with SPSS software (version 11.0, SPSS Inc., Chicago, IL, USA). Chi-square or Fisher's exact tests were used when comparing frequencies between groups. All numerical data are expressed as means ± SD, and differences between means of groups were compared by ANOVA test. Probability values less than 0.05 were considered statistically significant.

RESULTS

Clinicopathologic features

Nineteen (63.3%) of the 30 FTN-INPTCs arose on a background of NH, and 11 cases (36.7%) on FAs (Fig. 1). Of the 19 FTN-INPTCs on an NH background, eight (42.1%) showed diffuse, incomplete PTC-type nuclear changes, and 11 (57.9%) had focal nuclear changes. In contrast, of the 11 FTN-INPTCs on an FA background, that is, the encapsulated FTN-INPTCs, three (27.3%) showed the nuclear changes focally, and eight (72.7%) showed diffuse nuclear changes. Of the 30 FTN-INPTCs, preoperative aspiration cytology was performed in 12 cases. Three cases were diagnosed as benign nodules, 5 as follicular neoplasms, 3 as indeterminate and one as 'suspicious for papillary carcinoma'. Resected specimens were diagnosed as nodular hyperplasia in 12 cases, follicular adenoma in 8, atypical adenoma in 2, WDTUMP in 4 and FVPTC in the remaining 4 cases. Sixteen (53.3%) of the 30 FTN-INPTCs had concurrent PTC, compared with only two of the NHs and none of the FAs ($p<0.001$, Table 1). The ages of the patients tended to increase in the order: benign nodule, FTN-INPTC, and FVPTC ($p=0.077$, Table 1).

Expression of PTC-related markers

Immunohistochemical expression of galectin-3, HBME-1, CK19, fibronectin-1, and CITED1 differed significantly between the groups. Most of these markers were not expressed in the benign nodules, or, in a few cases, showed only focal reactivity (Table 2). Exceptionally, CK19 was expressed in five benign nod-

ules (14.3%, two cases of FA and three cases of NH), and the two FA cases showed diffuse expression of CK19. There was no significant difference between FTN-INPTCs with an NH background and those with an FA background in the expression of galectin-3 (52.6% vs 45.5%), CK19 (47.4% vs 27.3%), HBME-1 (100% vs 81.8%) or CITED-1 (78.9% vs 72.7%). Fibronectin-1 was expressed only in FTN-INPTCs with an FA background (36.4% vs 0%; p=0.005), and there was no significant difference in the expression of the markers between FTN-INPTCs with focal atypia and those with diffuse atypia. The expression of galectin-3, HBME-1, CK19, and CITED1 was significantly higher in FTN-INPTCs than in benign nodules (Table 2).

Table 1. Clinicopathologic features of benign nodules, FTN-INPTCs, and FVPTCs

	Benign nodule[a] (n=35)	FTN-INPTC (n=30)	FVPTC (n=26)
Age (year)	45.7±13.5	50.3±12.4	52.8±10.8
Male:Female	11:24	8:22	3:23
Presence of papillary carcinoma in other area	2 (5.7%)[b]	16 (53.3%)[b]	NA

[a]Benign nodules consist of 21 follicular adenomas and 14 nodular hyperplasias; [b]p<0.001, benign nodule vs FTN-INPTC.
FTN-INPTC, follicular patterned thyroid nodule with incomplete nuclear features of PTC; FVPTC, follicular variant of papillary thyroid carcinoma; NA, not applicable.

Comparing FTN-INPTCs with FVPTCs, the expression of galectin-3, CK19, and fibronectin-1 was significantly higher in FVPTCs (Table 2; Fig. 2, 3). All of the FVPTCs showed diffuse and strong expression of galectin-3, whereas 8 (53.3%) of

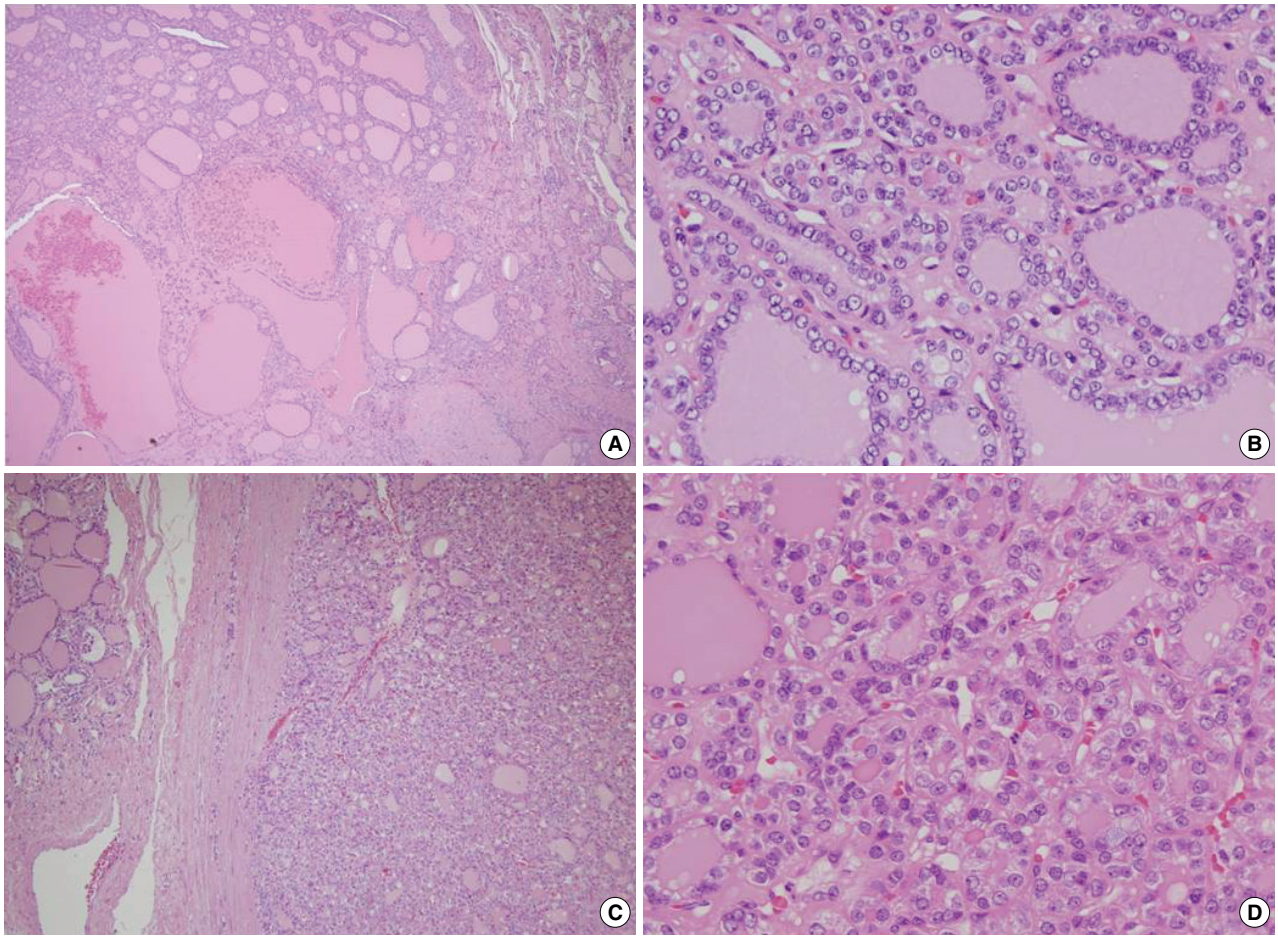

Fig. 1. A case of follicular patterned thyroid nodule with incomplete nuclear features of papillary carcinoma on a background of nodular hyperplasia (A, B). In a low magnification, this nodule has no capsule and shows marked size variation of follicles, resembling nodular hyperplasia (A). High power magnification reveals nuclear clearing and grooves, not sufficient for the diagnosis of papillary thyroid carcinoma (B). Another case of follicular patterned thyroid nodule with incomplete nuclear features of papillary carcinoma on a background of follicular adenoma (C, D). In a low magnification, this nodule has a well developed capsule and is composed of similar sized follicles, resembling follicular adenoma (C). High power magnification reveals nuclei with imperfect nuclear features of papillary thyroid carcinoma, showing minor degree of nuclear clearing and grooves (D).

15 galectin-3 positive FTN-INPTCs revealed diffuse but relatively week expression (p<0.001). While FVPTCs showed diffuse and strong staining to CK19 in a high proportion, FTN-INPTCs revealed only focal and weak staining (p<0.001). When comparing combined expression of these markers, all FVPTCs were positive for both galectin-3 and CK19, whereas six (20.0%) of 30 FTN-INPTCs were positive for both of them (p<0.001). And all of the six FTN-INPTCs revealed focal staining to CK19. Twenty (76.9%) of the 26 FVPTCs showed co-expression of galectin-3, CK 19 and fibronectin-1, while only two (6.7%) of the 30 FTN-INPTCs revealed their co-expression (p<0.001). There was no significant difference in HBME-1 and CITED1 expression between the two groups, despite some different patterns of expression: HBME-1 was diffusely expressed in all the FVPTC cases, whereas half of the FTN-INPTCs showed focal positivity for HBME-1, and the other half showed diffuse but inc-

Table 2. Immunohistochemical differences between benign nodules, FTN-INPTCs, and FVPTCs

Immunohistochemical marker pattern of expression	Benign nodule (n=35)	FTN-INPTC (n=30)	FVPTC (n=26)
Galectin-3	1 (2.9%)[a]	15 (50.0%)[a,d]	26 (100%)[d]
Diffuse:focal	0:1	8:7	26:0
HBME-1	2 (5.7%)[a]	28 (93.3%)[a,e]	26 (100%)[e]
Diffuse:focal	0:2	14:14	26:0
CK19	5 (14.3%)[b]	12 (40.0%)[b,d]	26 (100%)[d]
Diffuse:focal	2:3	0:12	21:5
Fibronectin-1	0 (0%)[c]	4 (13.3%)[c,d]	20 (76.9%)[d]
Diffuse:focal	0:0	0:4	12:8
CITED1	3 (8.6%)[a]	23 (76.7%)[a,f]	18 (69.2%)[f]
Diffuse:focal	0:3	6:17	7:11

[a]p<0.001; [b]p=0.027; [c]p=0.258, benign nodule vs FTN-INPTC; [d]p<0.001; [e]p=0.668; [f]P=0.531, FTN-INPTC vs FVPTC.
FTN-INPTC, follicular patterned thyroid nodule with incomplete nuclear features of PTC; FVPTC, follicular variant of papillary thyroid carcinoma.

Table 3. The distribution of *RASSF1A* PMR values in benign nodules, FTN-INPTCs, and FVPTCs

RASSF1A PMR	Benign nodule (n=35)	FTN-INPTC (n=30)	FVPTC (n=26)
0-1	2	0	0
1-3	8	1	4
3-4	3	3	0
4-5	6	2	2
5-10	6	9	4
10-50	9	11	14
>50	1	4	2
Methylation positive (PMR>4)	22 (62.9%)	26 (86.7%)	22 (84.6%)

FTN-INPTC, follicular patterned thyroid nodule with incomplete nuclear features of PTC; FVPTC, follicular variant of papillary thyroid carcinoma; PMR, percent of methylated reference.

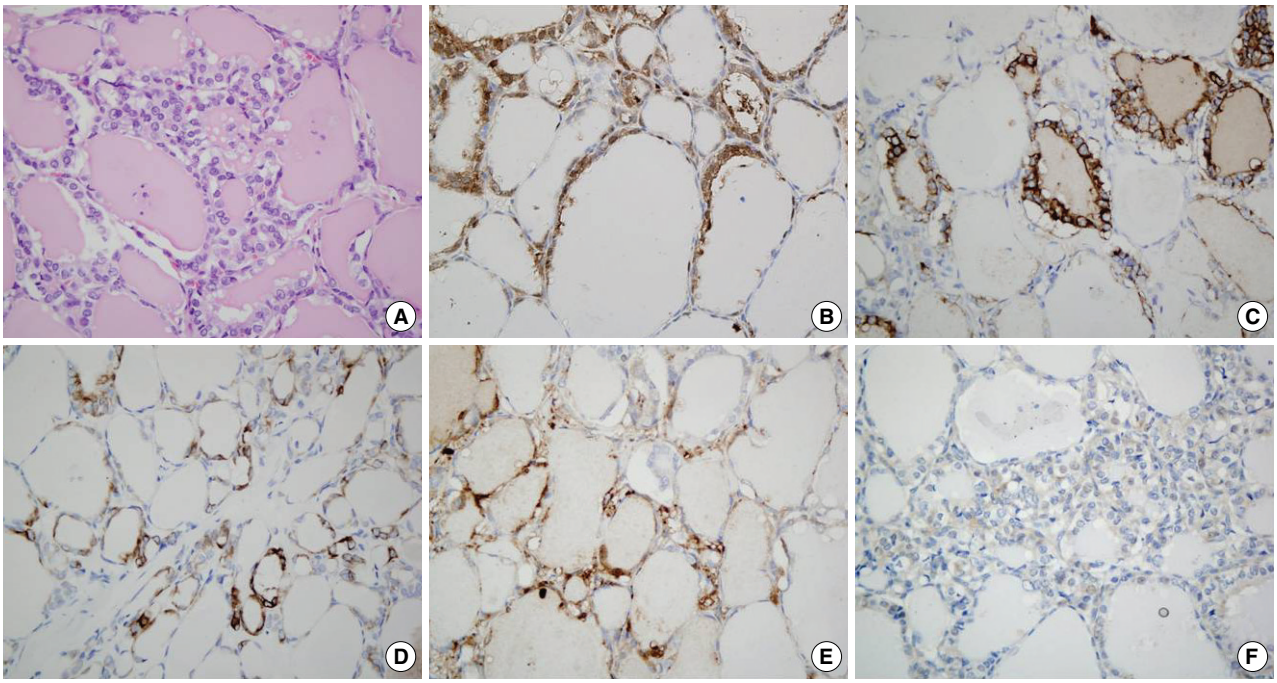

Fig. 2. A case of follicular patterned thyroid nodule with incomplete nuclear features of papillary thyroid carcinoma. Follicles contain irregular and clear nuclei resembling papillary carcinoma-type nuclear changes (A). The cells show focal expression of galectin-3 (B), HBME-1 (C), CK19 (D), and fibronectin-1 (E). There is weak cytoplasmic and nuclear staining for CITED1 (F).

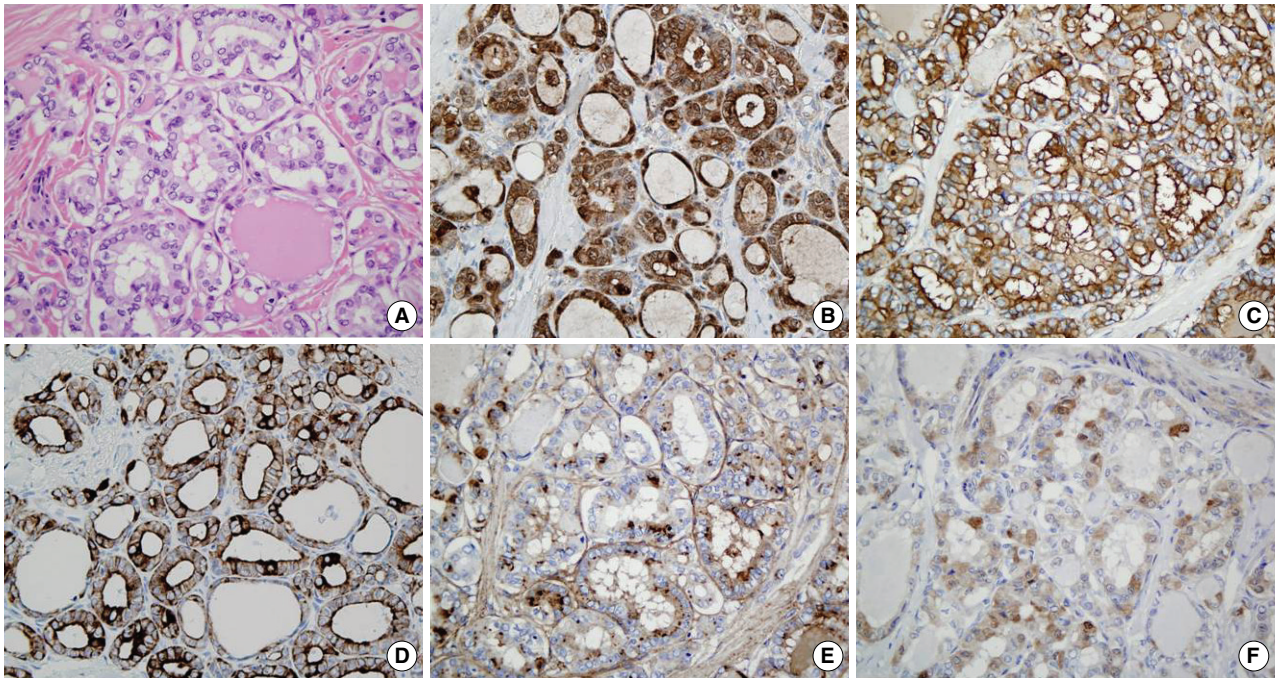

Fig. 3. A control case of follicular variant of papillary carcinoma (A). In this case, galectin-3 (B), HBME-1 (C) and CK19 (D) show diffuse expression. Fibronectin-1 (E) is focal but there is strong cytoplasmic expression, and CITED1 (F) shows focal cytoplasmic and nuclear expression.

omplete expression along the cytoplasmic membrane. CITED1 was expressed in a relatively high proportion of both FTN-INPTCs and FVPTCs (76.7% vs 69.2%).

BRAF mutation and RASSF1A promoter methylation

The *BRAF* V600E mutation was not found in any of the cases of benign nodules and FTN-INPTCs, but 12 (57.1%) of the 21 FVPTC harbored the *BRAF* V600E mutation.

The methylation rate of the RASSF1A promoter was significantly higher in FTN-INPTCs than in benign nodules (86.7% vs 62.9%; p=0.029; Table 3). However, there was no significant difference between FTN-INPTCs and FVPTCs, and in both groups there was a high rate of methylation in the *RASSF1A* promoter region (86.7% and 84.6%, respectively).

DISCUSSION

FTN-INPTC has been a subject of debate because of its potential for malignant transformation to PTC and several ambiguous aspects. A wide spectrum of intra- or interobserver variation in the diagnosis of FTN-INPTC has also been suggested,[19] and sometimes fixation artifacts seem to contribute to the morphological finding of PTC-like nuclei. Due to these complicating factors, FTN-INPTC has occasionally been overdiagnosed as FVPTC, despite incomplete nuclear changes, or underdiagnosed as a benign nodule including FA or NH, particularly in cases with focal nuclear atypia. FTN-INPTC still poses the diagnostic problems for many pathologists as to whether it should be reported as benign, borderline, or malignancy such as FVPTC.

In encapsulated follicular lesions with incomplete nuclear features of PTC, the terms 'follicular adenoma' or 'WDTUMP' have been used.[1-3] However, incomplete nuclear features of PTC are also found in non-encapsulated benign nodules, such as NHs or Hashimoto thyroiditis.[4,20,21] Fusco *et al.* reported that poorly developed PTC-type nuclear changes were observed focally or diffusely in NHs and the *RET/PTC* rearrangement could be detected in these areas.[21] Other authors reported PTCs within hyperplastic nodules,[22] suggesting that some NHs are precursors of well-differentiated carcinomas. On the other hand, coexisting PTCs are well-documented in Hashimoto thyroiditis.[23] In addition, the frequent detection of galectin-3, HBME-1, and cyclin D1 expression, and of the *RET/PTC* rearrangement in PTC-type pale nuclei implies that these atypical lesions are predisposed to malignancy.[24,25] Therefore, we selected both encapsulated and non-encapsulated follicular lesions with incomplete nuclear features of PTC, and divided them into two groups: FTN-INPTC

with overall features of NH (non-encapsulated) or FTN-INPTC with overall features of FA (encapsulated). Cases of Hashimoto thyroiditis were excluded for several reasons; first, the sizes of the atypical foci containing PTC-type nuclear changes are usually too small to obtain sufficient tissue for analysis. Second, they contain a lot of intrinsic biotin that limits the reliability of the immunohistochemical results. Lastly, the presence of lymphocytes in Hashimoto thyroiditis would confuse interpretation of the methylation status of the lesion of interest.

Our results represent the borderline morphologic, immunohistochemical, and molecular characteristics of FTN-INPTC. Expression of galectin-3, CK19, HBME-1 and CITED1 was significantly higher in FTN-INPTCs than in benign nodules. Immunoexpression of galectin-3 and CK19 was not only significantly higher in FVPTCs than in FTN-INPTCs, but also more diffuse and intense. Expression of HBME-1 was incomplete in FTN-INPTCs, although it was highly expressed in both FVPTCs and FTN-INPTCs. Because results from the combination of these markers have been reported to be helpful in the differential diagnosis of PTC,[13,26] we compared combined expression of galectin-3, CK19 and fibronectin-1 in FVPTC and FTN-INPTCs. While most of the FVPTC showed co-expression of them, only a few cases of FTN-INPTCs showed their co-exexpression. Our immnohistochemical results seem to illustrate the general borderline features of FTN-INPTCs. They are also consistent with previous reports that have suggested that WDTUMP and non-encapsulated FTN-INPTC are intermediate lesions between malignancy and benign nodules.[4,11,15,27] We found no difference between the immunohistochemical findings for FTN-INPTCs with focal nuclear changes and those with diffuse nuclear changes, and the expression of markers was generally confined to areas with PTC-type nuclear changes. Previous workers have also demonstrated that thyroid nodules with foci showing PTC-like nuclear change are positive for these markers only in cells with PTC-like nuclear atypia.[27,28]

None of our 24 FTN-INPTC cases had the *BRAF* V600E mutation (vs 57.1% of FVPTCs), but *RASSF1A* promoter methylation was as frequent in FTN-INPTCs as in FVPTCs (86.7% and 84.6%, respectively). These findings are consistent with the recent report that the BRAF mutation is not present in WDTUMPs, i.e. the encapsulated form of FTN-INPTC.[22] On the other hand, in studies of *RAS* mutations and the *RET/PTC* rearrangement, WDTUMPs and non-encapsulated FTN-INPTCs were found to be intermediate between benign nodules and carcinoma.[21,28] Interestingly, *RET* activation closely parallels the morphological changes; in other words, the molecular alterations are restricted to areas featuring PTC-like nuclei. The absence of the *BRAF* V600E mutation and the high frequency of *RASSF1A* promoter methylation in our FTN-INPTC cases suggest that the *BRAF* mutation acts in the later stage of malignant transformation to PTC, whereas *RASSF1A* promoter methylation is an early event. Recent data support this hypothesis by showing that *RASSF1A* promoter methylation is present in both benign and malignant thyroid tumors, and that the *BRAF* mutation is restricted in PTCs and anaplastic carcinomas.[29]

The *BRAF* V600E mutation rate has been reported to be low in FVTPCs, being 32% at most.[30] Our FVPTC cases had a higher *BRAF* mutation rate (57.1%), which may partially reflect geographical factors since the *BRAF* mutation is common in Asian populations.[7]

In our study, concurrent PTC was more frequent in FTN-INPTCs compared to benign nodules (53.3% vs 5.7%; $p< 0.001$). This finding suggests that FTN-INPTC may arise from the thyroid gland predisposed to develop PTC and indicate the possibility of a coexisting PTC elsewhere in the thyroid gland.

To sum up, FTN-INPTC showed intermediate characteristics between the FVPTC and the benign nodule immunohistochemically. Moreover, *RASSF1A* methylation was significantly more frequent in FTN-INPTCs than in benign nodules, and the BRAF mutation was not observed in FTN-INPTCs. Therefore, FTN-INPTC should be distinguished from FVPTC or benign nodules. Although an appropriate diagnostic term for FTN-INPTC should be proposed, the term "WDTUMP" may be provisionally used for these cases irrespective of encapsulation status. Our immunohistochemical and molecular findings seem to reinforce the ambiguous nature of FTN-INPTCs; however, they suggest that the incomplete PTC-type nuclear changes observed by light microscopy are not artifacts but point to the potential for transformation into PTCs and that FTN-INPTCs are pathogenetically related to FVPTCs.

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