Noninvasive Follicular Thyroid Neoplasms With Papillary-like Nuclear Features Are Genetically and Biologically Similar to Adenomatous Nodules and Distinct From Papillary Thyroid Carcinomas With Extensive Follicular Growth

Daniel N. Johnson, MD; Larissa V. Furtado, MD; Bradley C. Long, BS; Chao Jie Zhen, BS; Michelle Wurst, MB, MLS (ASCP)/CM; Ibro Mujacic, MS; Sabah Kadri, PhD; Jeremy P. Segal, MD, PhD; Tatjana Antic, MD; Nicole A. Cipriani, MD

Context.—Proposed noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTPs), formerly noninvasive encapsulated papillary carcinoma, follicular variant (PTC-FV), is an indolent tumor with follicular growth and frequent RAS mutations.

Objective.—To detect histologic and molecular differences separating NIFTP from follicular adenomas (FAs) and invasive carcinomas, particularly papillary carcinomas with extensive follicular growth (PTC-EFGs) and invasive encapsulated PTC-FV (IE-PTC-FV).

Design.—Sixty-one tumors were reviewed histologically and reclassified into 32 NIFTPs (52%), 4 IE-PTC-FVs (7%), 14 PTC-EFGs (23%), and 11 FAs (18%). Next-generation sequencing for mutations in 50 genes was performed. Clinical outcomes were recorded.

Results.—The NIFTPs and FAs were well circumscribed and unencapsulated. The FAs had bland nuclei, whereas the NIFTPs showed at least 2 of 3 (67%; sufficient) nuclear features (enlargement, irregular contours, chromatin clearing). The IE-PTC-FVs had follicular growth, sufficient nuclear features, and extensive capsular invasion. The PTC-EFGs had a median of 5% papillae with intrathyroidal invasion (broad-based, sclerotic, or small follicle growth patterns); intranuclear pseudoinclusions were present only in PTC-EFGs (9 of 14; 64%). Mutations included RAS in 20 of the 32 NIFTPs (62%), 4 of the 11 FAs (36%), and 3 of the 4 IE-PTC-FVs (75%); BRAF V600E in 1 NIFTP (3%); BRAF V600E in 5 PTC-EFGs (36%). No NIFTPs or FAs recurred or metastasized. All 4 IE-PTC-FVs (100%) had hematogenous metastasis. Two PTC-EFGs (14%) had lymphatic metastasis.

Conclusions.—The morphologic similarity and RAS mutations in FAs, NIFTPs, and IE-PTC-FVs supports the genetic similarity of those follicular neoplasms in contrast to the unique presence of BRAF V600E mutations in PTC-EFGs. Using strict diagnostic criteria supported by molecular testing, tumors with extensive follicular growth can be classified into follicular type or RAS-like (FA, NIFTP, IE-PTC-FV) versus papillary type or BRAF V600E-like (PTC-EFG).

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radioactive iodine therapy are not recommended for its treatment.14

The use of NIFTP as a diagnostic entity attempts to clarify the controversial term infiltrative variant of papillary thyroid carcinoma (PTC-FV), which was first used to describe infiltrative neoplasms composed predominantly of follicles and with a sclerosing pattern of invasion, nuclear features, and lymph node metastasis, similar to classic PTC.15 Since then, nuclear features have superseded architectural features and behavior, and PTC-FV has become a heterogeneous and confusing entity. However, PTC-FV can be clarified and subclassified into 3 main groups, as described in a recent review by Tallini et al16:

1. An infiltrative type with sclerotic invasion, at least focal papillae, and a behavior similar to classic PTC, with frequent extrathyroidal extension and lymph node metastases, and BRAF V600E (but not RAS) mutations (as described by Chem and Rosai15 in 1977 and often called the infiltrative follicular variant of PTC). An unencapsulated follicular variant is recognized by the World Health Organization (WHO), which also has a natural history similar to classic PTC17.

2. A well-circumscribed type that may be partially or completely encapsulated, is composed almost entirely of follicles, lacks invasion, behaves indolently, and has RAS (but not BRAF V600E) mutations (called the noninvasive encapsulated follicular variant of PTC).4,5,18

3. An encapsulated type that is composed almost entirely of follicles and shows capsular, vascular, or intrathyroidal invasion; has the capacity for hematogenous metastases; and has RAS (but not BRAF V600E) mutations (called the invasive encapsulated follicular variant of PTC).

A diffuse follicular variant has been reported, rarely, in the literature; however, that variant has a variable prognosis and unknown genetics and is not within the scope of the current study.19,20

Given the heterogeneous and controversial nature of PTC-FV, and in light of the recognition of NIFTP by the WHO,17 we evaluated all subtypes of PTC-FV historically diagnosed at our institution and compared their morphologic and genetic features to benign follicular adenomas (FAs). We hypothesized that there would be discernible, histologic differences and distinct molecular abnormalities that could be used to separate NIFTPs, FAs, and invasive carcinomas.

### MATERIALS AND METHODS

With institutional review board approval, our institution’s diagnostic surgical pathology archives were searched for all cases of papillary thyroid carcinoma follicular variant between 2009 and 2016. Forty-four cases originally diagnosed as PTC-FV were identified and available. Two comparison groups were also selected: 11 benign FAs (also termed adenomatous/adenomatoid nodules by some pathologists because the clonal status of the benign nodules has little diagnostic or clinical import) and 13 cases diagnosed as classic papillary thyroid carcinoma with extensive follicular growth (PTC-EFG). That term was often used at our institution for infiltrative follicular variants to eliminate possible confusion with encapsulated follicular variants by treating physicians and/or patients. If benign FAs were present in sections containing a malignant diagnosis, they were also tested: 6 FAs were obtained from thyroids with PTCs in the opposite lobe, and 1 was obtained from a patient with a PTC in the same lobe but the opposite pole. The remaining 4 were obtained from consecutive thyroidectomies within 1 month, of which had a classic PTC in the opposite lobe (not part of this study).

All diagnostic hematoxylin-eosin–stained (H&E) tissue sections of all nodules were reviewed and reclassified by 3 pathologists into 1 of 4 diagnoses:

1. A benign (FA) diagnosis was rendered if the nodule demonstrated follicular growth, circumscription with no evidence of invasion, and insufficient nuclear features as proposed for NIFTP (see below).17

2. A diagnosis of NIFTP was rendered if the nodule showed circumscription with no evidence of invasion (capsular, vascular, or intrathyroidal), less than 1% true papillae (defined as complex, arborizing papillae with fibrovascular cores . . . not associated with a fine-needle aspiration lobe), less than 30% solid growth, absence of necrosis/increased mitosis/psammoma bodies, and presence of at least 2 of 3 (67%; ie, sufficient) nuclear features, defined as abnormalities in size/shape (enlargement, elongation, overlapping), membrane contour (irregular, grooved), or chromatin (clearing, margination, glassiness). Other than recommended “extensive” sampling, specific requirements for adequate evaluation of the periphery of NIFTP have not been agreed upon.13,17 Therefore, the adequacy of sampling was left to the discretion of the reviewing pathologists.

3. A diagnosis of invasive encapsulated PTC-FV (IE-PTC-FV) was rendered if the nodule had less than 1% true papillae, sufficient nuclear features were present, and demonstrated invasion. Because the WHO does not define invasion in encapsulated PTC-FV with invasion, we adopted all definitions of invasion for follicular carcinomas: minimally invasive (encapsulated with capsular invasion only; and angioinvasion and extrathyroidal invasion absent), encapsulated angioinvasive (encapsulated with angioinvasion; capsular invasion present or absent; and extrathyroidal invasion absent), or widely invasive (capsular or intrathyroidal invasion and extrathyroidal invasion; and angioinvasion present or absent).17

4. A diagnosis of invasive PTC-EFG was rendered if the nodule demonstrated at least 80% follicular growth, sufficient nuclear features were present, and demonstrated intrathyroidal invasion (in the form of broad-based nests, small irregular follicles, or sclerosis). Because the ratio of follicles to papillae in infiltrative and unencapsulated PTC-FV is not defined by the WHO, we selected 80 as a minimum percentage of follicles and adopted the unifying term PTC-EFG.17

Tumor-rich areas were identified on H&E sections, and the corresponding formalin-fixed, paraffin-embedded tissue blocks were retrieved. Genomic DNA was isolated from microdissected, tumor-enriched, formalin-fixed, paraffin-embedded tissue using the QiAamp DNA tissue kit (Qiagen, Valencia, California), according to manufacturer’s instructions. Quality DNA could not be isolated from 7 of 44 PTC-FVs (16%). These tumors were, therefore, excluded from the study, leaving 37 total PTC-FVs. Following extraction, DNA was quantified using the Qubit fluorometric assay (Thermo Fisher Scientific, Waltham, Massachusetts) and was further assessed for quantity and quality using a quantitative polymerase chain reaction assay (hgDNA Quantification and QC kit, F. Hoffmann-La Roche, Basel, Switzerland). Using a clinically validated assay, formalin-fixed, paraffin-embedded DNA from all samples was amplified for somatic mutations located within mutational hotspot regions of 50 cancer-related genes (Supplemental Table 1; see supplemental digital content containing 2 tables at www.archivesofpathology.org in the July 2018 table of contents) using multiplex polymerase chain reaction reagents (Roche). Polymerase chain reaction products were quantitated using the Qubit assay and were then used as a template to prepare the library for next-generation sequencing (HTP Library Preparation Kit, Roche), with selected, patient-specific, adapter index sequences. Libraries were quantified using a quantitative polymerase chain reaction assay (Library Quantification Kit, Roche) and
| Case No. | Nodule No. | Age, y/ Sex | Stage at Presentation | Nodule Size, cm | RAI Status | F/U Time, mo | F/U Status | Original Dx | Reclassified Dx | Gene With Pathogenic Mutation | cNomen | pNomen |
|----------|------------|-------------|-----------------------|-----------------|------------|--------------|------------|-------------|----------------|--------------------------------|---------|--------|
| 12       | a          | 30/F        | N/A                   | 2               | None       | 0.3          | NED        | FA          | FA             | HRAS<sup>c</sup>              | c.181C>A  | p.Q61K  |
| 9        | a          | 59/F        | N/A                   | 5.3             | None       | 0.4          | NED        | FA          | FA             | KRAS<sup>c</sup>              | c.35G>T   | p.G12V  |
| 11       | d          | 54/M        | pT1a(m), NX           | 4.0             | e          | 40.2         | NED        | FA          | FA             | NRAS<sup>c</sup>              | c.182A>G  | p.Q61R  |
| 14       | f          | 62/M        | pT1b, NX              | 2               | e          | 2.4          | NED        | FA          | FA             | c                 | N/A      | N/A    |
| 6        | a          | 71/F        | N/A                   | 2               | None       | 29.3         | NED        | FA          | FA             | None               | N/A      | N/A    |
| 13       | a          | 54/M        | pT1a, N0              | 5.2             | None       | 3.5          | NED        | FA          | FA             | None               | N/A      | N/A    |
| 16       | a          | 43/F        | pT1b(m), NX           | 6.8             | None       | 3.6          | NED        | FA          | FA             | None               | N/A      | N/A    |
| 19       | a          | 46/F        | pT1b(m), NX           | 11              | None       | 22.8         | NED        | FA          | FA             | None               | N/A      | N/A    |
| 22       | a          | 62/F        | pT1b, NX              | 6.1             | None       | 13.5         | NED        | FA          | FA             | None               | N/A      | N/A    |
| 25       | a          | 68/F        | pT1b, NX              | 2               | None       | 12.9         | NED        | PTC-FV      | NIFTP           | STK11               | c.802_853 | p.G268F8*10|
| 28       | a          | 45/F        | pT1b(m), NX           | 2               | None       | 9.6          | NED        | FA          | FA             | None               | N/A      | N/A    |
| 31       | a          | 43/F        | pT1b, NX              | 11              | None       | 4.0          | NED        | FA          | FA             | None               | N/A      | N/A    |
| 34       | a          | 47/F        | pT1b(m), NX           | 1.5             | None       | 4.0          | NED        | FA          | FA             | None               | N/A      | N/A    |
| 37       | a          | 60/F        | pT1b, NX              | 2               | None       | 2.1          | NED        | FA          | FA             | None               | N/A      | N/A    |
| 40       | a          | 57/F        | pT1b, NX              | 2               | None       | 2.1          | NED        | FA          | FA             | None               | N/A      | N/A    |
| 43       | a          | 53/F        | pT1b, NX              | 2               | None       | 1.9          | NED        | FA          | FA             | None               | N/A      | N/A    |
| 46       | a          | 65/F        | pT1b, NX              | 11              | None       | 2.6          | NED        | FA          | FA             | None               | N/A      | N/A    |
| 49       | a          | 68/F        | pT1b, NX              | 2               | None       | 2.3          | NED        | FA          | FA             | None               | N/A      | N/A    |

NIFTP: Similar to Adenoma, Unlike PTC—Johnson et al
| Case No. | Nodule No. | Age, y/ Sex | Stage at Presentation | Nodule Size, cm | RAI Status | F/U Time, mo | F/U Status | Original Dx | Reclassified Dx | Gene With Pathogenic Mutation | cNomen | pNomen |
|---------|------------|-------------|-----------------------|----------------|------------|--------------|------------|-------------|-----------------|-------------------------------|---------|--------|
| 28      | a          | 61/F        | pT2(m), NX, M1        | 2.4            | 126.6 mCi $^{131}$I oral | 30.0        | AWD, bony mets | PTC-FV       | IE-PTC-FV       | HRAS c.182A>G, p.Q61R       | p.61R   |         |
| 29      | a          | 64/F        | pT2, NX, M1           | 3.3            | 126.6 mCi $^{131}$I oral | 29.8        | AWD, lung mets | PTC-FV       | IE-PTC-FV       | HRAS c.182A>G, p.Q61R       | p.61R   |         |
| 29      | a          | 64/F        | pT2, NX, M1           | 3.3            | 126.6 mCi $^{131}$I oral | 29.8        | AWD, lung mets | PTC-FV       | IE-PTC-FV       | HRAS c.182A>G, p.Q61R       | p.61R   |         |
| 41      | a          | 57/F        | pT3(m), N1            | 8.7            | None initially; 200 mCi $^{131}$I oral | 37.3        | AWD, lung mets | PTC-EFG       | IE-PTC-FV       | KRAS c.35G>C, p.G12A        |         |         |
| 22      | a          | 64/M        | pT1b, NX, M1          | 1.4            | 152.5 mCi $^{131}$I oral | 45.7        | AWD, bony mets | PTC-FV       | IE-PTC-FV       | BRAF c.1799T>A, p.V600E     |         |         |
| 16      | a          | 50/M        | pT2, NX               | 2.6            | UNK        | 2.4          | NED        | PTC-FV       | PTC-FG          | BRAF c.1799T>A, p.V600E     |         |         |
| 32      | a          | 58/F        | pT1a, N0              | 0.9            | None       | 21.4         | NED        | PTC-FG       | PTC-FG          | BRAF c.1799T>A, p.V600E     |         |         |
| 36      | a          | 47/F        | pT1a, N0              | 0.9            | None       | 31.6         | NED        | PTC-FG       | PTC-FG          | BRAF c.1799T>A, p.V600E     |         |         |
| 39      | a          | 47/M        | pT3, N1a              | 2              | UNK        | 2.0          | NED        | PTC-FG       | PTC-FG          | BRAF c.1799T>A, p.V600E     |         |         |
| 40      | a          | 31/F        | pT2(m), N1a           | 2.8            | UNK        | 0.6          | NED        | PTC-FG       | PTC-FG          | BRAF c.1799T>A, p.V600E     |         |         |
| 35      | a          | 60/F        | pT1a,m,N0             | 0.4            | None       | 36.0         | NED        | PTC-FG       | PTC-FG          | PTC-FG c.1799T>A, p.V600E   |         |         |
| 3       | a          | 32/F        | pT1a,m, NX            | 0.5            | None       | 43.4         | NED        | PTC-FG       | PTC-FG          | PTC-FG c.1799T>A, p.V600E   |         |         |
| 13      | a          | 41/F        | pT1a, N0              | 0.4            | None       | 3.5          | NED        | PTC-FG       | PTC-FG          | PTC-FG c.1799T>A, p.V600E   |         |         |
| 4       | 1          | 62/M        | pT2, NX               | 2.2            | UNK        | 13.5         | NED        | PTC-FG       | PTC-FG          | PTC-FG c.1799T>A, p.V600E   |         |         |
| 19      | a          | 43/M        | pT1b, N0              | 1.7            | UNK        | 0.4          | NED        | PTC-FG       | PTC-FG          | PTC-FG c.1799T>A, p.V600E   |         |         |
| 31      | a          | 64/F        | pT1a,m,N0             | 1              | None       | 17.2         | NED        | PTC-FG       | PTC-FG          | PTC-FG c.1799T>A, p.V600E   |         |         |
| 33      | a          | 53/F        | pT1b,m,N0             | 1.8            | None       | 39.7         | NED        | PTC-FG       | PTC-FG          | PTC-FG c.1799T>A, p.V600E   |         |         |
| 37      | a          | 36/F        | pT1b,m,N0             | 1.9            | None       | 7.1          | NED        | PTC-FG       | PTC-FG          | PTC-FG c.1799T>A, p.V600E   |         |         |
| 51      | a          | 65/F        | pT2, N0               | 2.5            | UNK        | 0.2          | NED        | PTC-FG       | PTC-FG          | PTC-FG c.1799T>A, p.V600E   |         |         |

Abbreviations: AWD, alive with disease; cNomen, complementary DNA–level nomenclature; Dx, diagnosis; FA, follicular adenoma; F/U, follow-up; IE-PTC-FV, invasive encapsulated follicular variant of papillary thyroid carcinoma; N/A, not applicable; NED, no evidence of disease; NIFTP, noninvasive follicular thyroid neoplasm with papillary-like nuclear features; pNomen, protein-level nomenclature; PTC, papillary thyroid carcinoma; PTC-EFG, papillary thyroid carcinoma with extensive follicular growth; PTC-FV, papillary thyroid carcinoma follicular variant; RAI, radioactive iodine; UNK, unknown.

* Solitary nodule.
  * Metastatic nodule.
  * Nodule also had variant of uncertain clinical significance (see Supplemental Table 2).
  * Patient had PTC-classical not tested in this series.
  * Patient had RAI for concurrent carcinoma.
  * Patient had NIFTP tested in this series.
  * Patient had PTC-EFG tested in this series.

Eight patients with NIFTP (reclassified from PTC-FV) were originally diagnosed with multifocal carcinomas; 3 with incidental papillary microcarcinomas (patients 1, 45, and 47), 3 with grossly identified papillary carcinomas separate from the NIFTP (patients 8, 34, and 38), and 2 with "multifocal PTC-FV arising in a larger nodule" contemporarily considered to represent variable nuclear change in the single, larger nodule (patients 2 and 7). Therefore, the latter 2 carcinomas were no longer considered multifocal.
were then pooled and sequenced with an Illumina MiSeq (San Diego, California) system (2 × 152-base pair [bp] paired-end sequencing). Clinically validated bioinformatics analysis was performed on a Health Insurance Portability and Accountability Act–compliant, high-performance computing system (Center for Research Informatics, University of Chicago, Chicago, Illinois) using an in-house–developed bioinformatics pipeline, which includes quality checks (FastQC, Babraham Bioinformatics, Babraham, Cambridge, United Kingdom),21 alignment to the hg19 (GRCh37) human genome reference sequence, with primer and adapter trimming (NovoAlign, Novocraft, Selangor, Malaysia) and variant calling. Point mutations and small indels were detected using a combination of SAMtools mpileup22 and an in-house–developed bioinformatics toolkit to produce variant allele frequencies. Amplicon Indel Hunter 23 was used for supplementary detection of larger indels. Variant detection was performed at a threshold of 5% mutant allelic frequency (MAF). The resulting variants were annotated using Alamut Batch software (Interactive Biosoftware, Rouen, France).24 Additional filters were used on the annotated files, based on population variant frequencies25,26 (to remove inherited single-nucleotide polymorphisms) and protein coding effects, to return a final list of somatic variants that were used for interpretation.

Patients’ electronic medical records were reviewed for date of surgical resection, postsurgical treatment, date of most-recent follow-up, and disease status at follow-up.

RESULTS

Reclassification

After exclusion of 7 lesions with inadequate DNA, 61 nodules from 54 patients were studied (Table 1). The original diagnoses were 37 PTC-FVs (61%), 13 PTC-EFGs (21%), and 11 benign FAs (18%). The diagnosis of all 11 FAs (100%) remained unchanged. The diagnosis of 11 PTC-EFGs (85%) remained unchanged; 1 (8%) was reclassified to NIFTP; 1 (8%) was reclassified to IE-PTC-FV. The 37 PTC-FVs were reclassified to NIFTP (n = 31; 84%), PTC-EFG (n = 3, 8%), or IE-PTC-FV (n = 3, 8%). After reclassification, diagnoses were 11 FAs (18%), 32 NIFTPs (52%), 4 IE-PTC-FVs (7%), and 14 PTC-EFGs (23%).

Histologic Features

FAs.—Of the 11 benign FAs, 6 (55%) were submitted in their entirety for histologic evaluation; 1 to 2 sections/cm were submitted on the remainder. Eight FAs (73%) occurred in patients with other tumors: 4 of 11 patients (36%) with NIFTPs (patients 24, 45, 49, and 52), 3 patients (27%) with PTC-EFGs (patients 4, 13, and 36), and 1 patient (9%) with a classic PTC not tested (patient 11). Seven FAs were in lobes opposite the index lesion, and 1 was in the same lobe (opposite pole) as the index lesion (patient 13). All 11 FAs were well circumscribed but unencapsulated (Figure 1, A) and were composed of predominantly microfollicles (n = 4, 36%), predominantly macrofollicles (n = 2, 18%), or follicles of mixed sizes (n = 5, 45%). Cells were flattened to cuboidal and contained minimal to moderate amorphophilic to clear cytoplasm and small, dark, round nuclei, with finely stippled chromatin (Figure 1, B and C). Three (27%) showed oncocytic morphology with moderate to abundant eosinophilic cytoplasm and variably enlarged nuclei, with occasional prominent nucleoli or membrane irregularities, in keeping with oncocytic metaplasia (but insufficient to meet NIFTP criteria). Occasionally, random endocrine atypia was observed (Figure 1, D).

NIFTPs.—Of the 32 NIFTPs, 25 (78%) were submitted in their entirety for histologic evaluation; 4 (12%) had 2 to 3 cassettes/cm submitted; 3 (9%) had 1 to 2 cassettes/cm submitted (7 cassettes from a 5-cm tumor, 2 cassettes from a 1.9-cm tumor, and 1 cassette from 1.1-cm tumor). The 32 NIFTPs were well circumscribed, with thin to absent capsules (Figure 2, A) and composed of predominantly microfollicles (n = 16; 50%; Figure 2, B through D), predominantly macrofollicles (n = 8; 25%; Figure 2, E), or follicles of mixed sizes (n = 8; 25%). Cells were plump and contained minimal to moderate amorphophilic to eosinophilic cytoplasm. Three (9%) had abundant eosinophilic cytoplasm (Figure 2, D). Nuclei were always minimally to moderately enlarged with nuclear membrane irregularities (including grooves and/or indentations; Figure 2, D), nuclear vacuolation (so-called pseudo-pseudoinclusions; Figure 2, F and G), and/or marginated chromatin causing nuclear clearing (Figure 2, B). Twelve lesions (38%) met 2 of the 3 nuclear criteria for NIFTP, whereas the remaining 20 (62%) met all 3 nuclear criteria. None (0%) had psammoma bodies, true intranuclear cytoplasmic pseudo-inclusions, or capsular, vascular, or intrathyroidal invasion. Thyroids from 8 patients with NIFTP were originally diagnosed with multifocal carcinoma: 3 with incidental papillary microcarcinomas found in sections from grossly normal thyroid, 3 with grossly identified classic papillary carcinomas (0.8–1.1 cm) geographically separate from the NIFTP, and 2 with multifocal PTC-FVs arising in a larger nodule, which we currently consider to reflect variable nuclear change within the single, larger nodule. The latter 2 carcinomas were, therefore, no longer considered multifocal (Table 1). None had multiple NIFTPs.

IE-PTC-FVs.—The first IE-PTC-FV (patient 28) was submitted in its entirety for histologic evaluation and was composed predominantly of microfollicles with some macrofollicles. Papillae were not present. It had a calcified capsule and areas of tumor outside the capsule, consistent with capsular invasion (Figure 3, A). There was no definite angioinvasion or extrathyroidal invasion. Nuclei were irregular, cleared, and enlarged (Figure 3, B and C). An incidental papillary thyroid microcarcinoma was also present in grossly normal thyroid. The second IE-PTC-FV (patient 41) had predominantly macrofollicles filled with pale colloid and demonstrated transcapsular invasion and extrathyroidal extension into soft tissue (Figure 3, D). Hyperplastic-appearing papillae were present, including the formation of Sanderson polsters bulging into large follicles (Figure 3, E). Cells were oncocytic with abundant eosinophilic cytoplasm, enlarged nuclei, occasionally prominent nucleoli, irregular nuclear membranes, and chromatin clearing (Figure 3, E, bottom inset). Psammoma bodies were also identified (Figure 3, E, top inset). One section per centimeter of tumor was submitted. An encapsulated, noninvasive follicular variant of PTC was also present in the opposite pole of the same lobe (not tested in this series). The third IE-PTC-FV (patient 29) consisted of mixed-sized follicles without true papillae. It showed a small, encapsulated component with extensive tumor present outside the capsule, consistent with capsular invasion (Figure 3, F). There was no definite angioinvasion or extrathyroidal invasion. Nuclei were enlarged with irregular contours (Figure 3, G). Two sections per centimeter of tumor were submitted. The fourth IE-PTC-FV (patient 22) was submitted in its entirety for histologic evaluation and contained microfollicles with an extensively fibrotic edge and intraleSIONAL fibrosis, which effaced a large proportion of tumor cellularity (Figure 3, H). Cells had eosinophilic to clear cytoplasm, nuclei showed enlargement, overlapping and
irregular membranes, and mitotic activity was present (Figure 3, I and J). Foci suspicious for angioinvasion were identified; however, there was no obvious capsular or extrathyroidal invasion. None of the IE-PTC-FVs had histologic features of poorly differentiated or undifferentiated (anaplastic) transformation.

**PTC-EFGs.**—Of the 14 PTC-EFGs, 10 (71%) were entirely submitted for histologic evaluation; 1 to 3 sections/cm were submitted on the remainder. All demonstrated evidence of intrathyroidal invasion in the form of either large nests of broad-based invasion (n = 3; 21%; Figure 4, A), small follicle invasion into adjacent normal thyroid (n = 7; 50%; Figure 4, B), or sclerosing invasion with abundant, associated fibrosis (n = 4; 29%; Figure 4, C). On average, they had 6% papillae (range, <1 to 20%; median, 5; mode, 5; Figure 4, D) with follicles predominantly microfollicular (n = 6; 43%), macrofollicular (n = 3; 21%), or mixed (n = 5; 36%). Cells were enlarged and contained moderate to abundant eosinophilic to amphophilic cytoplasm. Nuclei were always enlarged, with chromatin clearing and/or nuclear membrane irregularities, including grooves and irregular contours (Figure 4, E). Intranuclear cytoplasmic pseudoinclusions were present in 9 cases (64%; Figure 4, F and G). Three cases (21%) had psammoma bodies. All 3 cases (100%) with less 1% papillae had true pseudoinclusions; 2 (67%) showed small follicle, and 1 (33%) showed sclerosing invasion.

**Next-Generation Sequencing.**—In the reclassified groups, 4 of 11 benign FA (36%) had a RAS mutation (2 NRAS, 1 each HRAS and KRAS). None had a BRAF mutation. Twenty of the 32 NIFTPs (62%) had a RAS mutation (11 NRAS, 6 HRAS, 3 KRAS); 1 NIFTP (3%) had a BRAF K601E mutation. Two NIFTPs (6%; both with RAS mutations) harbored additional, likely pathogenic mutations in SMAD4 and STK11 (1 case each). Three of 4 IE-PTC-FVs (75%) had a RAS mutation (1 each NRAS, HRAS, and KRAS). None had a BRAF mutation. Five of 14 PTC-EFGs (36%) had a BRAF V600E mutation. None (0%) had pathogenic RAS mutations. The remaining tumors had no pathogenic mutations (Tables 1 and 2).

All 14 pathogenic NRAS mutations occurred in the hotspot codon 61 (either Q61K [n = 3; 21%] or Q61R [n = 11; 79%]). Pathogenic HRAS mutations were Q61R (4 of 8; 50%), Q61K (n = 2; 25%), or G13R (n = 2; 25%). Pathogenic...
Figure 2. Histology of noninvasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTP). A, Predominantly microfollicular nodule with a well-circumscribed border and an NRAS Q61R mutation (patient 18). B, Most NIFTPs met all 3 nuclear criteria, as in this nodule (patient 20). C, Some tumors met 2 of the 3 nuclear criteria (often enlargement and grooves without clearing), as in this nodule (patient 14). D, Three NIFTPs demonstrated abundant eosinophilic cytoplasm and large nuclei with irregular contours, chromatin clearing, and nucleoli (arrow). An NRAS Q61R mutation was found in this nodule (patient 52). E, A macrofollicular NIFTP with an HRAS G13R mutation (patient 47). F and G, Some NIFTP had nuclear vacuoles or “pseudo-pseudoinclusions” showing blurry, pale centers without crisp inner membranes (arrow), as in these KRAS Q61R-mutated nodules (patient 46 [F]; patient 54 [G]) (hematoxylin-eosin, original magnifications ×40 [A] and ×600 [B through G]).
KRAS mutations were G12V (2 of 5, 40%), G12A (n = 1, 20%), or Q61R (n = 2, 40%). Patient 54 harbored a complex insertion-deletion mutation in exon 6 of STK11, resulting in a frameshift of the protein coding sequence and creating a premature stop codon. The MAF was 3.2% with a read coverage of 444 (compared with 14.6% MAF and a read coverage of 5159 for the concurrent KRAS mutation), raising the possibility of a subclonal mutational event/intratumoral heterogeneity. Although less than the 5% MAF threshold, this variant was included because it passed manual inspection of the sequencing reads. In addition, STK11 mutations have been detected in rare cases of follicular thyroid carcinoma (FTC), tall cell variant of papillary carcinoma, and encapsulated follicular variant of PTC, although their specific role in thyroid carcinogenesis is incompletely understood. Patient 5 harbored a SMAD4 R361H mutation (5.7% MAF and a read coverage of 1377, compared with 42.4% MAF and a read coverage of 1848 for the concurrent NRAS mutation), raising the possibility of a subclonal event/intratumoral heterogeneity. Although less than the 5% MAF threshold, this variant was included because it passed manual inspection of the sequencing reads. In addition, STK11 mutations have been detected in rare cases of follicular thyroid carcinoma (FTC), tall cell variant of papillary carcinoma, and encapsulated follicular variant of PTC, although their specific role in thyroid carcinogenesis is incompletely understood. Patient 5 harbored a SMAD4 R361H mutation (5.7% MAF and a read coverage of 1377, compared with 42.4% MAF and a read coverage of 1848 for the concurrent NRAS mutation), raising the possibility of a subclonal event/intratumoral heterogeneity. Although less than the 5% MAF threshold, this variant was included because it passed manual inspection of the sequencing reads. In addition, STK11 mutations have been detected in rare cases of follicular thyroid carcinoma (FTC), tall cell variant of papillary carcinoma, and encapsulated follicular variant of PTC, although their specific role in thyroid carcinogenesis is incompletely understood. Patient 5 harbored a SMAD4 R361H mutation (5.7% MAF and a read coverage of 1377, compared with 42.4% MAF and a read coverage of 1848 for the concurrent NRAS mutation), raising the possibility of a subclonal event/intratumoral heterogeneity. Although less than the 5% MAF threshold, this variant was included because it passed manual inspection of the sequencing reads. In addition, STK11 mutations have been detected in rare cases of follicular thyroid carcinoma (FTC), tall cell variant of papillary carcinoma, and encapsulated follicular variant of PTC, although their specific role in thyroid carcinogenesis is incompletely understood. Patient 5 harbored a SMAD4 R361H mutation (5.7% MAF and a read coverage of 1377, compared with 42.4% MAF and a read coverage of 1848 for the concurrent NRAS mutation), raising the possibility of a subclonal event/intratumoral heterogeneity. Although less than the 5% MAF threshold, this variant was included because it passed manual inspection of the sequencing reads. In addition, STK11 mutations have been detected in rare cases of follicular thyroid carcinoma (FTC), tall cell variant of papillary carcinoma, and encapsulated follicular variant of PTC, although their specific role in thyroid carcinogenesis is incompletely understood.

Recent clinicopathologic and molecular studies have shown that the well-circumscribed, noninvasive types of PTC-FV are indolent tumors with little to no capacity for metastasis and a predominance of RAS mutations, similar to follicular neoplasms. The term NIFTP has been proposed for lesions meeting strict diagnostic criteria. Labeling these lesions as tumors, rather than carcinomas, more accurately reflects their biologic potential and promotes less-aggressive patient management, that is, no need for completion thyroidectomy or radioactive iodine therapy. That change will likely positively affect patient quality of life and alleviate potential psychologic ramifications of a cancer diagnosis. We evaluated tumors previously diagnosed as PTC-FV and compared their histologic appearance and molecular genetics to benign FAs and papillary thyroid carcinomas with extensive follicular growth (Table 2). Most PTC-FV (31 of 37; 84%) met diagnostic criteria for NIFTPs. In our institution, the qualifying terms well-circumscribed or encapsulated were infrequently used during the study period; instead, PTC-FV often implied a noninvasive tumor. Therefore, our reclassification rate from PTC-FV to NIFTP may be higher than other institutions. Six PTC-FVs (6 of 37; 16%) remained invasive carcinomas; 3 classic PTC-EFGs (50%) and 3 IE-PTC-FVs (50%). The diagnosis of all benign nodules remained unchanged, as did most of the cases originally diagnosed as PTC-EFGs (11 of 13; 85%); 1 (8%) met criteria for NIFTP, and 1 (8%) was reclassified to IE-PTC-FV.

**DISCUSSION**

Recent clinicopathologic and molecular studies have shown that the well-circumscribed, noninvasive types of PTC-FV are indolent tumors with little to no capacity for metastasis and a predominance of RAS mutations, similar to follicular neoplasms. The term NIFTP has been proposed for lesions meeting strict diagnostic criteria. Labeling these lesions as tumors, rather than carcinomas, more accurately reflects their biologic potential and promotes less-aggressive patient management, that is, no need for completion thyroidectomy or radioactive iodine therapy. That change will likely positively affect patient quality of life and alleviate potential psychologic ramifications of a cancer diagnosis.

We evaluated tumors previously diagnosed as PTC-FV and compared their histologic appearance and molecular genetics to benign FAs and papillary thyroid carcinomas with extensive follicular growth (Table 2). Most PTC-FV (31 of 37; 84%) met diagnostic criteria for NIFTPs. In our institution, the qualifying terms well-circumscribed or encapsulated were infrequently used during the study period; instead, PTC-FV often implied a noninvasive tumor. Therefore, our reclassification rate from PTC-FV to NIFTP may be higher than other institutions. Six PTC-FVs (6 of 37; 16%) remained invasive carcinomas; 3 classic PTC-EFGs (50%) and 3 IE-PTC-FVs (50%). The diagnosis of all benign nodules remained unchanged, as did most of the cases originally diagnosed as PTC-EFGs (11 of 13; 85%); 1 (8%) met criteria for NIFTP, and 1 (8%) was reclassified to IE-PTC-FV.

Of the final 32 NIFTPs, 20 (62%) had RAS mutations and 1 (3%) had a BRAF K601E mutation, which has been found in FAs, PTCs, and encapsulated PTC-FVs. This relatively rare BRAF mutation is considered RAS-like because of its prevalence in follicular neoplasms, association with indolent
Figure 3. Histology of invasive encapsulated follicular variants of papillary thyroid carcinoma. A, An HRAS Q61R–mutated carcinoma (patient 28) with a calcified capsule and macrofollicular tumor component (lines) on the outside of the capsule. B and C, This tumor showed nuclear enlargement, membrane irregularities, chromatin clearing, and occasional mitoses (arrow). D, A KRAS G12A–mutated carcinoma (patient 41) with transcapsular invasion by tumor cells and an extrathyroidal extension into adjacent skeletal muscle. E, In areas, hyperplastic-appearing papillae with thick, fibrous cores branched into large follicles, creating Sanderson polsters (arrow). This carcinoma had psammoma bodies (top inset) and enlarged, cleared nuclei (bottom inset). F, An NRAS Q61K–mutated carcinoma (patient 29) showing a large, invasive tumor mass outside the capsule (arrow) of a
behavior in noninvasive tumors, and a mechanism of activation of the MAPK and PI3K signaling pathways distinct from that of BRAF V600E mutated tumors.\(^1\,13,32,33\) No case of NIFTP had the BRAF V600E mutation. No case of NIFTP recurred or metastasized, although 12 of 32 (37.5%) received postoperative radioactive iodine. Twelve of 32 NIFTPs (37.5%) had 2 of 3 nuclear features for NIFTP, and the other 20 (62.5%) had all 3 criteria. No case of NIFTP had true intranuclear cytoplasmic pseudoinclusions or psammoma bodies. Many cases had so-called pseudo-pseudoinclusions, which are not cytoplasmic invaginations into the nucleus but are vacuolated nuclei secondary to processing artifacts.\(^3\) Two NIFTPs (6%) were subcentimeter tumors (0.4 and 0.7 cm in patients 5 and 34); both of which had the RAS mutation. All NIFTPs in the Nikiforov et al.\(^{\text{13}}\) proposal were larger than 1 cm, and smaller tumors have not been systematically studied.\(^3\) Subcentimeter, noninvasive, encapsulated PTC-FVs may also be considered part of the NIFTP spectrum because their morphology, genetics, and behavior are congruent.\(^6\,36\) Lastly, 8 NIFTPs (25%) were larger than 4 cm (range, 4.1–8.1 cm, in patients 17, 25, 52, 1, 20, 48, 2, and 44); 3 of which had RAS mutation, consistent with published data supporting inclusion of larger nodules into NIFTP.\(^3\)

Of the final 14 PTC-EFGs, 5 (36%) had BRAF V600E mutation; no case had pathogenic RAS mutation. Two tumors metastasized to regional lymph nodes; both cases had the BRAF V600E mutation. Morphologically, all showed evidence of intrathyroidal invasion. Eleven cases (79%) had true papillae (ranging from 1% to 20%; median, 5%). True evidence of intrathyroidal invasion. Eleven cases (79%) had the nuclear features of PTC, and not associated with a fine-needle aspiration area.

The Cancer Genome Atlas study.\(^{13}\) Grouping those into NIFTP spectrum because their morphology, genetics, and behavior are congruent.\(^6\,36\) Lastly, 8 NIFTPs (25%) were larger than 4 cm (range, 4.1–8.1 cm, in patients 17, 25, 52, 1, 20, 48, 2, and 44); 3 of which had RAS mutation, consistent with published data supporting inclusion of larger nodules into NIFTP.\(^3\)

Four cases of invasive carcinoma were classified as IE-PTC-FVs. Three (75%) showed invasion outside the tumor capsule (1 additionally with extrathyroidal invasion). One case (25%) showed likely angioinvasion and extensive peritumoral and intratumoral fibrosis that would not be expected in a benign neoplasm. Although not classic capsular invasion, that growth pattern was hypothesized to represent an unusual form of invasion or regression. None had true neoplastic papillae. One case (25%) had hyperplastic papillae and 1 (25%) had psammoma bodies. Three (75%; including those with hyperplastic papillae and psammoma bodies) had RAS mutations, and all had hematogenous (not lymphatic) metastasis, supporting their biologic behavior as follicular or RAS-like (rather than papillary or BRAF V600E-like) neoplasms.\(^2,\,8\,10,\,39,\,40\) RAS mutations identical to the primaries were found in visceral metastases in the 2 tested cases; metastases in the other 2 cases were not tested. In the presence of “sufficient” nuclear features, invasive neoplasms with follicular growth might be deemed invasive encapsulated follicular variants of PTC; however, that term could be confusing and may not fully reflect their genetic or behavioral similarity to FTCs.\(^{13}\) Some authors advocate a diagnosis of FTC with nuclear atypia.\(^2,\,41\) Similarly, The Cancer Genome Atlas study\(^1\) concluded that reclassification of such tumors was warranted because they were among the RAS-like carcinomas and were distinct from BRAF V600E-like PTCs. Although molecular and clinical data are supportive, consensus is still needed regarding the nomenclature of these tumors.

Demonstrated in this and other studies is the rate of RAS mutation (36%; 4 of 11) in benign follicular adenomatous nodules with bland nuclei.\(^{42,\,43}\) Those nodules were chosen because they were all well circumscribed, composed entirely of follicles, and lacked thick capsules. High rates of clonality have been found in adenomatous/hyperplastic nodules (suggesting that they are true neoplasms), even though they are histologically indistinguishable from nonclonal counterparts.\(^{45,\,50}\) Morphologically, the greatest difference between the FAs and the NIFTPs was the degree of papillary-like nuclear change. Genetically, behaviorally, and histologically, these 2 groups are similar. RAS mutations were found in 1 of 4 (25%) FAs with a concurrent NIFTP (1 with the same NRAS Q61K mutation), 1 of 4 (25%) FAs with a concurrent PTC, and 2 of 3 (67%) FAs without concurrent tumors. Therefore, we hypothesize that neoplastic FAs arise as independent clonal events unrelated to tumorigenesis of other neoplasms within the same thyroid gland.

Some patients with tumors reclassified as NIFTPs (n = 12; 38%) received radioactive iodine treatment because of their original diagnosis of carcinoma (PTC-FV). It cannot be known whether those would have metastasized or recurred in the absence of such therapy. However, the clinical behavior of the NIFTPs remains distinct from that of the IE-PTC-FVs and PTC-EFGs. Using the same diagnostic criteria, we and others have shown that untreated NIFTPs have not recurred or metastasized.\(^{13,\,36}\) In addition, although the number of NIFTPs we were able to test was relatively moderate (32), our numbers were slightly more or similar to...
Figure 4. Histology of classic papillary thyroid carcinomas with extensive follicular growth (PTC-EFG). A, A carcinoma (patient 33) with broad-based, intrathyroidal invasion in the form of large nests with an undulating border. B, A carcinoma (patient 4) with invasion in the form of small follicles (arrows) adjacent to nearby bland, nonneoplastic follicles. C, A BRAF V600E–mutated carcinoma (patient 39) showing the sclerosing pattern of invasion (abundant dense collagen admixed with tumor) into perithyroidal adipose tissue. D, True papillae with fine fibrovascular cores lined with neoplastic cells (arrows) in a different BRAF V600E–mutated carcinoma (patient 40). E, In other areas, the same carcinoma showed small follicle formation with enlarged, wrinkled, cleared nuclei. F and G, True intranuclear, cytoplasmic pseudo-inclusions with crisp inner membranes and eosinophilic contents (arrows) were seen in 64% of PTC-EFGs (patient 51 [F]; patient 40 [G]) but in no noninvasive follicular thyroid neoplasms with papillary-like nuclear features (hematoxylin-eosin, original magnifications ×20 [A and C], ×200 [B and D], and ×600 [E through G]).
other studies.\textsuperscript{4,5,9,11} Our numbers were limited primarily by the relative rarity of PTC-FV and occasionally by difficulty in isolating quality DNA from thyroid tumors (7 PTC-FV), which were, therefore, excluded from this study. The number of IE-PTC-FVs tested was particularly limited (n = 4) likely because of its even greater rarity, as well as by the design of our study (in which we did not search for FTCs with possible nuclear atypia).

Unfortunately, sequencing for thyroid carcinoma-associated translocations or TERT promoter mutations (possibly associated with aggressive behavior) was not available at our institution at the time of this study. However, sequencing of TP53 and PIK3CA (found in poorly differentiated and anaplastic thyroid carcinomas\textsuperscript{50}) was performed, and mutation was not identified in any tumor. The purpose of this study was not to evaluate for, or to predict, aggressive behavior based on mutation. Instead, it was designed to correlate morphologic features with RAS-like versus BRAF-like genetics. Another limitation of our study is the use of the proposed nuclear scoring system for NIFTP, which has not yet been prospectively validated. However, we did find that the nuclear scoring system could not differentiate between encapsulated follicular neoplasms with and without pathogenic RAS-like mutations. Of all 24 noninvasive, encapsulated follicular neoplasms (FAs or NIFTPs) with such mutations, 4 (17%) did not have sufficient nuclear features and were considered FAs. That low percentage is likely due to the relatively fewer tested FAs (n = 11) compared with NIFTPs (n = 32) and would be expected to increase with inclusion of additional FAs. Interobserver variability in evaluation of nuclear features (and, therefore, in diagnosis of FA versus NIFTP) further blurs the line between those 2 entities.

Our findings coincide with those reported regarding the behavior and genetics of noninvasive, encapsulated follicular thyroid carcinoma (NIFTP) versus infiltrative PTC (PTC-EFG).\textsuperscript{1,4,6,9,13,12} Some authors suggest that encapsulated, noninvasive PTC-FVs (NIFTPs), because of their expected benign behavior, are best thought of as FAs with papillary-like nuclear features or atypia.\textsuperscript{2,52} In this context, we contribute to the expanding body of knowledge by demonstrating histologic, genetic, and behavioral similarity of NIFTPs to benign FAs (never, to our knowledge compared in the same study before), and histologic, genetic, and behavioral similarity of IE-PTC-FV to FTC. We also report the first cases of identical RAS mutations detected in both primary and metastatic IE-PTC-FVs, further supporting this distinction and the RAS-like phenotype of IE-PTC-FVs. Our findings lend credence to the concept that IE-PTC-FVs may be conceptualized as FTCs with nuclear atypia and NIFTPs as FAs with nuclear atypia.\textsuperscript{2,52} Use of the term papillary-like nuclear features may overemphasize the similarity of those neoplasms to papillary carcinoma, which we and others have shown to be dissimilar. Increased emphasis on differences in overall architecture could lead to better understanding and diagnosis of equivocal cases. Some hypothesize that NIFTPs are preinvasive neoplasms with the ability to transform to carcinomas and should be given appropriate weight.\textsuperscript{33}

Unfortunately, the natural history of follicular neoplasms (FAs or NIFTPs) is not well understood. The rate of malignant transformation of benign neoplasms is unknown; however, when cytologically benign nodules have been followed over time, development of malignancy is rare.\textsuperscript{33} Whether NIFTPs (or even FAs) represent precursors to invasive carcinoma has yet to be determined. Considering NIFTPs as FAs or follicular neoplasms with altered nuclei reflects their genetic origins and likely indolent behavior, acknowledging that the transformative potential of RAS-driven neoplasms (with or without altered nuclei/NIFTP nuclear features) is not thoroughly understood.

In conclusion, we favor use of the descriptive diagnosis papillary thyroid carcinoma with extensive follicular growth (PTC-EFG), as opposed to the variant diagnoses infiltrative or unencapsulated PTC-FV, which may help eliminate possible confusion in use of the term follicular variant. Additionally, using strict diagnostic criteria supported by molecular testing, tumors of follicular growth can be accurately classified into 1 of 2 categories: follicular-type or RAS-like tumors (including FAs, NIFTP/FAs with atypia, and IE-PTC-FVs/FTCs with atypia) versus papillary-type or BRAF V600E-like tumors (PTC-EFG).

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