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Review Article

Thyroid tumors with follicular architecture

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ABSTRACT

Thyroid tumors with follicular architecture encompass a considerable array of distinct entities. These lesions share significant morphologic overlap, but portend different prognostic and therapeutic implications. Due to their similar growth patterns, distinction between these tumors can be difficult; remarkable interobserver variability exists, even between expert endocrine pathologists. Given the diagnostic challenges associated with these lesions, establishment of the correct diagnosis requires adequate gross examination protocol, careful attention to morphologic features and pathologic context, as well as—increasingly—adjunct molecular findings. In this review, we summarize the salient features of various follicular thyroid tumors, with special emphasis on the recently defined category of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), as well as the molecular pathology of these lesions.

1. Introduction

Follicular-patterned tumors of the thyroid represent a broad spectrum of lesions, with a wide variety of cytologic features, molecular alterations, and clinical implications. These lesions share considerable morphologic overlap in many instances, and proper diagnosis of follicular tumors can accordingly pose significant challenges. Even among experts, several studies have demonstrated remarkable interobserver variability in several circumstances. These scenarios include, but are not limited to, diagnosis of encapsulated follicular lesions [1], and diagnosis of the follicular variant of papillary thyroid carcinoma (FVPTC) [2,3].

Interestingly, rates of some follicular tumors have changed over various periods, due to several factors. During the twentieth century, dietary iodide supplementation led to decreased incidence of follicular thyroid carcinoma (FTC) in many parts of the world. Despite this drop, however, pathologists frequently rendered this diagnosis erroneously, confusing FTC with benign lesions (e.g. partially encapsulated hyperplastic nodules, “pseudoinvasion” after fine needle aspiration, et cetera), as well as other malignancies, especially FVPTC [reviewed in 4]. This scenario illustrates the diagnostic difficulties associated with thyroid neoplasia showing follicular architecture.

Proper diagnosis of these lesions requires understanding of their biology, morphologic features, and—increasingly—their molecular pathology. In this review, we discuss the spectrum of follicular-patterned thyroid tumors. We place special emphasis on the relatively new category of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), as the definition and profile of this entity continue to evolve.

2. Follicular adenoma

Follicular adenoma (FA) is a benign neoplasm composed of follicular epithelial cells. The tumor shows three hallmark histologic features: follicular architecture; distinct patterning, relative to the uninvolved parenchyma; and encapsulation, or at least circumscription separating it from non-neoplastic tissue (Fig. 1A). These properties facilitate distinction from follicular adenomatoid (hyperplastic) nodules, which exhibit architectural variability, incomplete (if any) circumscription, and generally do not form discrete, demarcated lesions distinguishable from adjacent parenchyma [reviewed in 5]. FA may comprise microfollicular, normofollicular/eufollicular, or macrofollicular architecture (Fig. 1B). Trabecular, insular, or solid areas may be present [6], as these growth patterns are not independently diagnostic of poorly differentiated thyroid carcinoma. The fibrous capsule of FA is generally thin but may be thick (Fig. 1C), and shows neither capsular nor vascular invasion. As the absence of invasive growth differentiates FA from FTC, any follicular neoplasm may be regarded as potentially malignant until these findings are ruled-out. This prospect requires adequate gross sampling and histologic examination in order to exclude malignancy [7]. Prior studies have shown that thorough examination of the capsular surface enables distinction between adenoma and carcinoma [8]. Most contemporary authors advocate submission of the entire capsule (one group recommended examination of at least 10 sections of capsular tissue, without explicitly requiring assessment of the entire surface) [9]. By definition, follicular adenoma lacks nuclear features of papillary thyroid carcinoma (PTC).

Several variants of FA have been described. FA with Hürthle cell features is discussed in a separate section of this review (please see

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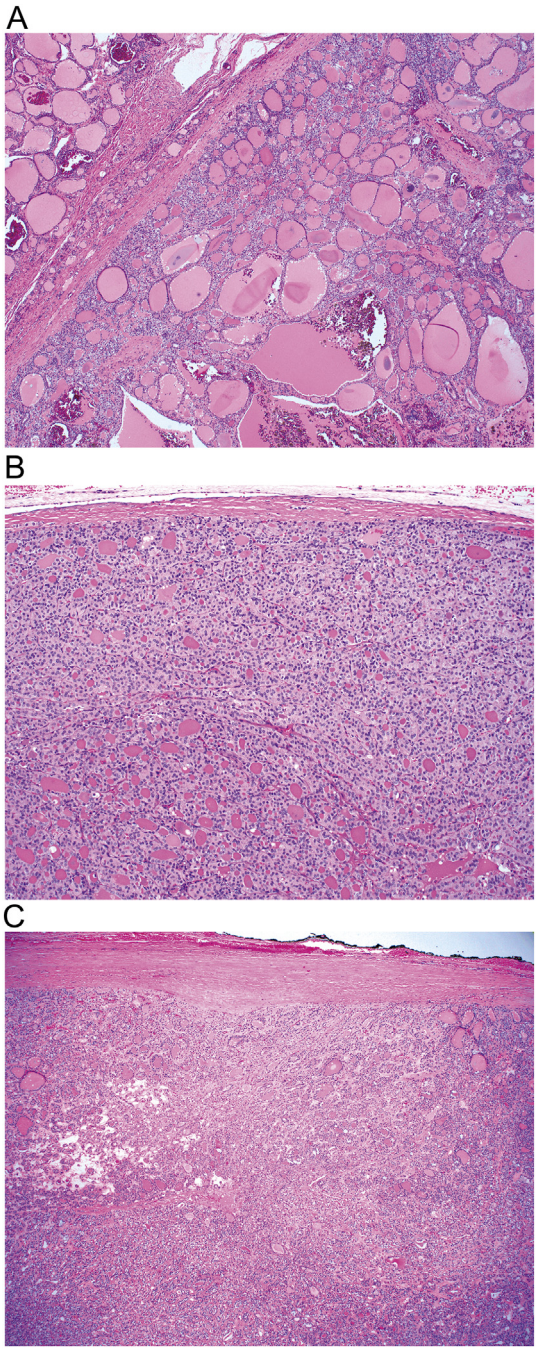

Fig. 1. Follicular adenoma. A. Follicular-patterned tumor separated from the adjacent parenchyma by a thin capsule (upper left). B. Follicular adenoma with microfollicular growth. C. Follicular adenoma with a thick capsule (top); capsular thickness is not independently diagnostic of malignancy.

below). Other predominant cell types that define FA variants include signet-ring cells (follicular adenoma, signet-ring cell variant) [10] and clear cells (follicular adenoma, clear cell variant) [11]. In a study of the signet-ring cell variant, one group proposed arrested folliculogenesis as the pathophysiologic mechanism, on the basis of positive thyroglobulin immunohistochemistry, negative mucin staining, and ultrastructural examination [10]. Many authors have reported fat-containing versions of FA, variably called fat-containing follicular adenoma, lipid-rich cell adenoma, lipoadenoma, and adenolipoma [12-15]. Evaluation of one "lipid-rich cell adenoma" demonstrated admixture of lipid, glycogen, and sudanophil crystals within the neoplastic cells. According to the authors, these findings implicate altered intracellular lipid metabolism, rather than abnormal lipid storage, in the tumor cells' appearance [14]. Autonomously functioning ("hot") FA has been described, including 13 cases among a series of 17 hot nodules [16]. Other, more exotic reported varieties include glomeruloid follicular adenoma [17].

Immunohistochemistry (IHC) is generally unnecessary to establish the diagnosis of FA, although IHC may be warranted in some circumstances, particularly when cases show variant morphology (e.g., signet-ring cell and clear cell variants). The neoplastic cells in FA, including all variants, demonstrate cytoplasmic staining for thyroglobulin, and nuclear staining for thyroid transcription factor 1 (TTF1) and PAX8. Staining for neuroendocrine markers (e.g. synaptophysin, chromogranin) is negative, as is staining for lineage-specific markers of the gastrointestinal tract (e.g. CDX2, SATB2) and kidney (e.g. renal cell carcinoma marker).

In terms of demographic associations, FA occurs more frequently among female patients. The tumor is generally a solitary lesion. Accordingly, many pathologists interpret the scenario of multifocal follicular nodules as hyperplastic, rather than synchronous adenomata [6]. Coexistence of multiple adenomata is rare, and warrants consideration of a genetic syndrome. Although FA is most often sporadic, the tumor occurs in the context of several inherited conditions. These include *PTEN* hamartoma tumor syndromes, such as Cowden Syndrome and Bannayan-Riley-Ruvalcaba Syndrome [18,19]. In one study, FA occurred in 25% of patients with these syndromes [20]. FA may also be seen in Carney complex, a multiple neoplasia syndrome associated with endocrine tumors, which results from germline mutations in *PRKAR1A* (protein kinase A regulatory subunit 1α) and other genes [21,22]. Radiation exposure represents another risk factor for FA (and other thyroid lesions), as demonstrated by evaluation of individuals affected by the Chernobyl accident, and of individuals exposed to "therapeutic" radiation [23,24]. Iodine deficiency is another risk factor, and the incidence of FA is higher in iodine-deficient areas [6].

2.1. Follicular carcinoma

Like FA, FTC arises from follicular epithelial cells, shows follicular patterning, and lacks nuclear features of PTC. FTC differs from FA by demonstrating invasive growth, i.e. capsular and/or vascular invasion. The capsule surrounding FTC is usually thicker, compared to that of FA [25]. FTC exhibits variable degrees of invasion, but authors describe the extent of invasion differently. According to the World Health Organization (WHO), FTC is either minimally invasive (defined as invasive into or through its capsule only), encapsulated angioinvasive, or widely invasive [25]. Other authors, however, include angioinvasive cases in the minimally invasive category [26]. In one classification scheme, minimally invasive FTC includes three scenarios: cases with (1) capsular invasion only; (2) limited vascular invasion (< 4 vessels); and (3) extensive vascular invasion (≥ 4 vessels). Another group proposed criteria for minimal invasion including "small-to-medium vessel invasion, capsular invasion of up to full thickness, no parenchymal tumor extension, and no tumor necrosis" [27]. As its name imparts, widely invasive FTC shows widespread growth into the adjacent parenchyma and extrathyroidal soft tissue. This form often presents with distant metastases [28].

For both capsular and vascular invasion (angioinvasion), distinction between bona fide infiltrative growth and various mimics can be difficult. Accordingly, rigid criteria are applied to the identification of these findings. Capsular invasion entails neoplastic cells penetrating the entire thickness of the tumor capsule (Fig. 2A, B). Vascular invasion requires tumor emboli within vessels located outside the tumor itself, i.e. vasculature within or beyond the fibrous capsule. Involvement of blood vessels within the cellular component of the tumor does not qualify as vascular invasion, as this finding is not associated with malignant behavior. In terms of the tumor emboli, diagnosis of vascular invasion is indicated when intravascular tumor cells are associated with fibrin thrombus formation, and/or adherent to the vessel wall and

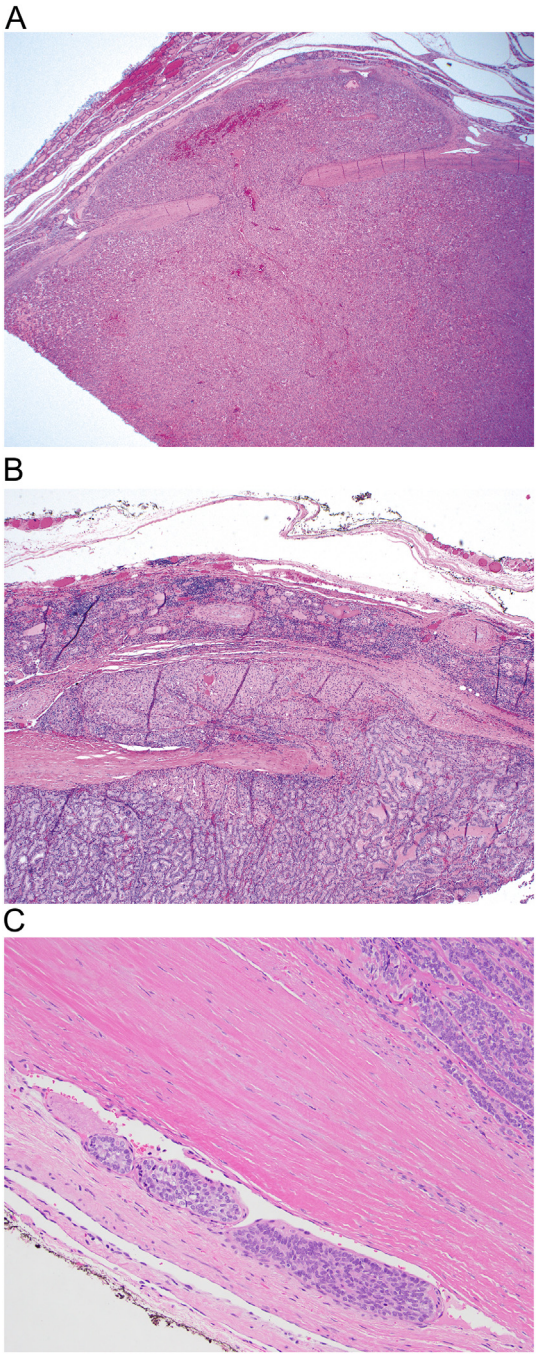

Fig. 2. Follicular carcinoma. A. Encapsulated follicular-patterned tumor showing mushroom-like protrusion of the tumor through its capsule. This finding represents capsular invasion, and is diagnostic of follicular carcinoma. B. Encapsulated follicular-patterned tumor with full-thickness capsular invasion, in which the tumor has bifurcated its capsule. C. Vascular invasion. Tumor embolus, associated with fibrin thrombus and partially covered by endothelium, adherent to the wall of an intracapsular blood vessel.

covered by endothelium (Fig. 2C). FTC metastasizes via hematogenous routes; lymphatic invasion and nodal metastasis occur rarely, if ever, and may warrant consideration of Hürthle cell (oncocytic) carcinoma, or FVPTC. As above, widely invasive carcinoma refers to the extent of parenchymal invasion, rather than vascular invasion [25].

Many variants of FTC are similar to variants of FA. These include FTC with fat cells, signet-ring cell FTC [10], FTC with a glomeruloid pattern [29], spindle cell FTC [30], and others. At least two examples of functioning FTC with associated hyperthyroidism have been reported [16,31]. Clear cell change has been reported in FTC; different authors variably describe this phenomenon as clear cell change versus a clear cell variant [11,32,33]. One group investigated the prognostic significance of this category, and determined that FTC with clear cell changes behaves analogously to its non-clear cell counterpart [33].

The immunohistochemical findings in FTC are the same as those for FA (see above).

Several factors affect the prognosis of FTC. In one large series of 292 patients, extensive vascular invasion (defined as 4 or more foci), age $\geq$45 years, and tumor size > 4 cm independently predicted shorter disease-free survival. Extensive vascular invasion and tumor size > 4 cm also independently increased likelihood of death from disease. Capsular invasion, however, showed no prognostic significance [26]. Multiple subsequent series have demonstrated little or no impact of capsular invasion on patient outcomes. Other studies have confirmed the adverse prognostic effect of extensive vascular invasion. One group evaluated the effect of vascular invasion for all encapsulated follicular cell-derived thyroid carcinomas (including encapsulated PTC, encapsulated FTC, and encapsulated Hürthle cell carcinoma). These investigators demonstrated extensive vascular invasion (again defined as $\geq$4 foci) as an independent predictor of poor recurrence-free survival. Conversely, patients with focal vascular invasion showed very low risk of recurrence, even without radioactive iodine treatment [34].

As with FA, risk factors for FTC include exposure to ionizing radiation [35] and insufficient dietary iodine. Authors in China have documented decreased incidence of FTC following implementation of universal salt iodization [36]. FTC also occurs in the context of genetic syndromes, including *PTEN* hamartoma tumor syndromes [19,20,37], Carney syndrome/Carney complex [21,38], and Werner syndrome (caused by mutation of the *WRN* gene) [39].

3. Hürthle cell (oncocytic) tumors

In the current WHO classification, Hürthle cell (oncocytic) tumors comprise a separate category of neoplasia, distinct from FA and FTC. This terminology is best applied when Hürthle cells constitute > 75% of a tumor, whereas lesions with fewer oncocytic cells are described as having Hürthle cell features [40]. Some pathologists use the terms Hürthle cell and oncocyte interchangeably. However, whereas an oncocyte is any cell with granular eosinophilic cytoplasm, Hürthle cells also have characteristic nuclear features including enlarged, round, central nuclei, generally with prominent nucleoli (Fig. 3). Architectural patterns of Hürthle cell tumors include follicular, trabecular, and solid. Hürthle cell carcinoma exhibits clinical behavior distinct from that of FTC. Whereas the latter virtually never metastasizes to lymph nodes,

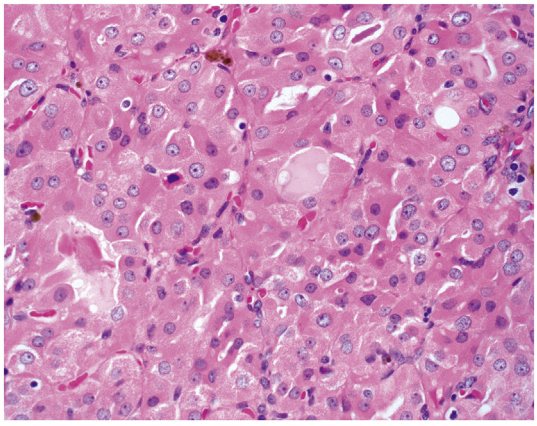

Fig. 3. Hürthle cell neoplasia, comprising cells with brightly eosinophilic granular cytoplasm. Note the enlarged and variably clear nuclei that are characteristic of oncocytic thyroid lesions (i.e., non-diagnostic of PTC in this context).

Hürthle cell carcinoma can spread via lymphatic and/or hematogenous routes [41]. In terms of other clinical features, Hürthle cell carcinoma occurs more frequently among men and among older patients, compared with other differentiated thyroid carcinomas [42].

The diagnostic criteria for Hürthle cell carcinoma (i.e. for capsular and vascular invasion) are the same as for FTC. Hürthle cells occur in a variety of contexts, including chronic lymphocytic (Hashimoto) thyroiditis, adenomatoid nodules, and FA. Consequently, the presence of Hürthle cells in fine needle aspiration biopsies (FNAB) or core biopsies is not diagnostic of malignancy. The increased mitochondrial content of Hürthle cells renders these tumors prone to reactive and degenerative changes following FNAB or core biopsy, including hemorrhage, infarction, and necrosis (individual cells and confluent foci). These secondary changes may complicate histologic evaluation of Hürthle cell lesions, and should not be misinterpreted as tantamount to malignancy. Rather, distinction between benign and malignant Hürthle cell neoplasia depends upon identification of capsular and/or vascular invasion, as with FA and FTC.

Pathologists and surgeons formerly regarded all Hürthle cell tumors as malignant, or at least potentially malignant. Investigators have shown that Hürthle cell carcinoma has more aggressive behavior, with lower overall and disease-specific survival, compared with other differentiated thyroid carcinoma [42]. In one early series of 62 patients with Hürthle cell neoplasia, 14 deaths were directly attributable to recurrent or metastatic disease; 3 of these 14 tumors had been diagnosed as benign. Accordingly, the authors advocated an aggressive surgical approach, involving total thyroidectomy or early completion thyroidectomy following lobectomy for all Hürthle cell tumors larger than 2 cm (as well as malignant tumors of any size). They achieved a lower recurrence rate (21% versus 59%) using this algorithm [43]. In subsequent studies, however, other authors demonstrated the capability to predict benign versus malignant clinical behavior based on morphologic criteria, similar to those used for FTC [44]. Other groups have assessed the prognostic significance of various pathologic parameters within Hürthle cell carcinoma. In one cohort, large tumor size and vascular invasion were associated with clinically aggressive tumors. Conversely, no patient without vascular invasion (i.e., no patient with capsular invasion only) developed metastatic disease. Furthermore, no patient with a tumor smaller than 3.5 cm developed metastatic disease, even when vascular invasion was present. These authors also presented evidence indicating Ki67 and cyclinD1 as useful for distinguishing Hürthle cell adenoma from carcinoma [45]. A different group showed that Hürthle cell tumors 4 cm or greater were malignant 65% of the time, and concluded that total thyroidectomy should be considered for patients with Hürthle cell tumors of this size and larger [46].

4. Follicular variant of papillary thyroid carcinoma

FVPTC is a carcinoma with follicular architecture, but with nuclear features of PTC. This diagnostic category has evolved over several decades [reviewed in 47]. In the mid-twentieth century, papillary and follicular carcinomas were defined and diagnosed according to their most prevalent growth pattern [48]. Further study showed that carcinomas with combined features of PTC and FTC showed biologic behavior more like that of PTC [49]. A pathologist first used the term “follicular variant of papillary carcinoma” in 1960, although he included this entity as one of three subgroups of FTC [50]. In a subsequent series of FVPTC, the authors demonstrated its morphologic and clinical similarity to PTC, supporting its classification as a variant of PTC rather than FTC [51].

By definition, FVPTC shows nuclear features of PTC. These features include enlargement and/or elongation of nuclei with crowding/overlapping, chromatin clearing, and irregular nuclear contours forming grooves and pseudoinclusions. The degree or caliber of nuclear changes interpreted as compatible with PTC, and required to establish this diagnosis, has dropped progressively over time [52,53]. The lowering of this diagnostic threshold corresponds to increased incidence rates of PTC, and increased ratios of PTC to FTC in the late twentieth and early twenty-first centuries, documented in multiple countries [54]. In addition to follicular patterning and PTC nuclei, FVPTC shows other characteristic (albeit non-diagnostic) features. These additional findings include strongly eosinophilic colloid within the lumina of neoplastic follicles [55], as well as the “sprinkling” sign, or patchy distribution of nuclear features throughout the tumor [56]. The latter is interesting and paradoxical (as well as potentially frustrating), since the focality and inconsistency of diagnostic findings is sufficiently reliable to be a characteristic feature, rather than an aberration.

Two major subtypes of FVPTC have been characterized: an infiltrative form, and an encapsulated form [57]. Some pathologists have described the former, when widely pervasive, as the diffuse or multinodular follicular variant, noting multicentricity (diffuse involvement of the whole gland, without formation of grossly discernible nodules), predilection for younger patients, and worse prognosis relative to unifocal, encapsulated FVPTC [58-60]. Authors have shown that infiltrative FVPTC spreads to lymph nodes like classical PTC, and more frequently entails extrathyroid extension and positive surgical margins [61]. Encapsulated FVPTC, however, behaves like FA or FTC (depending whether invasive growth occurs; see discussion of NIFTP below), with distant metastatic potential rather than metastasis to cervical nodes.

Molecular findings within these forms of FVPTC support the distinction between these respective varieties. FVPTC demonstrates a molecular signature different from that of classical PTC, and more akin to that of FA/FTC [62-64]. With higher granularity, one group showed that encapsulated FVPTC harbors high rates of *RAS* mutations, whereas they lack *BRAF* mutations, similar to FA/FTC. Infiltrative FVPTC, however, shares features of classical PTC molecular pathology, with the BRAF V600E mutation occurring more frequently than *RAS* alterations [61].

In addition to these two broad categories, several more unusual varieties of FVPTC have also been described. Rare cases of a macrofollicular variant have been reported, in which the neoplastic follicles are cystically dilated, simulating adenomatoid nodules (nodular hyperplasia). This variant is of particular interest, as it can be misinterpreted as a macrofollicular adenoma or nodular goiter, and may represent a source of diagnostic error [65-67]. In one series of 17 cases autonomously functioning (hot) nodules, the authors report one case of functioning FVPTC [16].

5. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features

Studies of encapsulated FVPTC have consistently demonstrated indolent behavior and low (if any) malignant potential in the absence of capsular or vascular invasion [reviewed in 47]. In one study, none of 43 patients with noninvasive, encapsulated FVPTC developed recurrences [57]. In another series, 16% of patients with invasive encapsulated FVPTC had poor outcomes, whereas none occurred in any noninvasive cases [68]. Other groups have similarly shown this entity's very low risk of metastasis or recurrence [69].

Accordingly, a working group within the Endocrine Pathology Society performed a study to address the malignant potential of encapsulated FVPTC. The working group retrospectively evaluated over 200 cases, separated into two groups. Group 1 included patients with noninvasive encapsulated FVPTC who received no radioiodine treatment, and at least 10 years of follow-up. This group consisted of 109 patients observed for 10 to 26 years, all of whom were alive with no evidence of disease. Group 2 included patients with encapsulated FVPTC showing capsular and/or vascular invasion, and at least one year of follow-up. This group consisted of 101 cases (80 cases with capsular invasion, 12 with vascular invasion, and 9 with both invasion types). Among patients in group 2, 12 experienced an adverse event

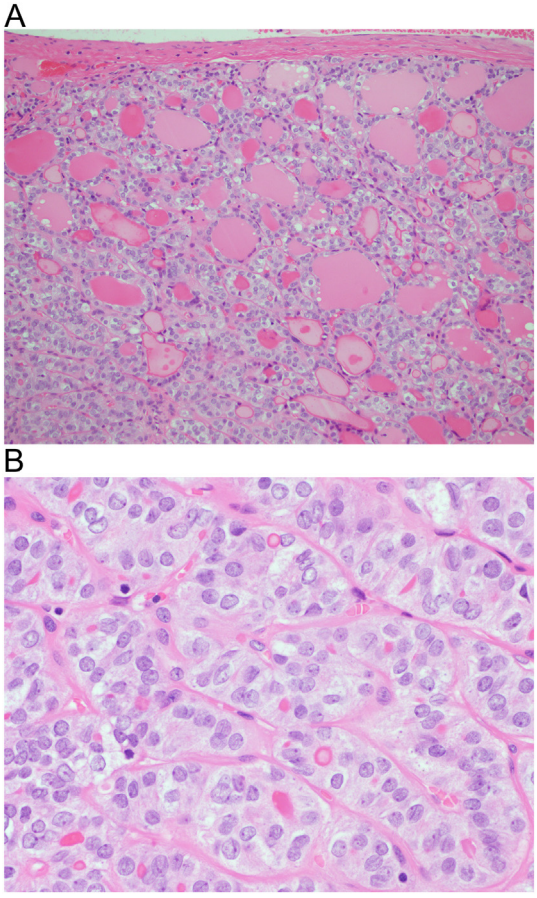

Fig. 4. Noninvasive follicular thyroid neoplasm with papillary-like features (NIFTP). A. Encapsulated follicular-patterned neoplasm, without evidence of capsular or vascular invasion (following evaluation of the entire capsular surface; one representative image shown). B. NIFTP with moderate PTC nuclear features (nuclear score 2). Variably enlarged, elongated, crowded nuclei, with finely dispersed chromatin and focal clearing, as well as occasional nuclear grooves. Presence of florid PTC nuclear features (nuclear score 3; not shown here) warrant examination of the entire tumor to exclude classical (or other) PTC.

over follow-up periods ranging from 1 to 18 years. Based on the different outcomes between patients with invasive and noninvasive tumors, the working group proposed new terminology for noninvasive encapsulated FVPTC: “noninvasive follicular thyroid neoplasm with papillary-like nuclear features” (NIFTP) [70].

This diagnosis is subject to rigid criteria, in order to reduce the likelihood of including carcinoma cases with higher potential to metastasize and/or recur. First, NIFTP applies to tumors with complete or partial encapsulation, as well as unencapsulated tumors that are well demarcated from adjacent, non-neoplastic parenchyma. Second, NIFTP lacks capsular and vascular invasion. Third, the tumor comprises follicular patterning with < 1% true papillary architecture (according to the original definition), although up to 30% trabecular/insular/solid growth is permitted (Fig. 4A). Fourth, the lesion shows nuclear features of PTC, although these are typically subtle, and their distribution ranges from diffuse to patchy. The authors devised a quantitative scoring algorithm to determine whether a lesion satisfies criteria for PTC-type nuclear features. Diagnostic nuclear changes were separated into three categories: (1) irregularities of size and shape (enlargement/elongation, overlapping/crowding); (2) irregularities of membrane contour (grooves, pseudoinclusions); and (3) irregularities of chromatin characteristics (clearing with margination, glassy nuclei). A pathologist assigns a score of 0 or 1 for each of these categories, yielding a total score that ranges from 0 to 3. Cases with a nuclear score of 2–3 fulfill criteria for NIFTP (or PTC). NIFTP cases typically receive a total score of 2, i.e. expression of PTC nuclear features is moderate (Fig. 4B).

Exclusion criteria are applicable as well. Findings that are incompatible with NIFTP include > 1% true papillary architecture, psammoma bodies, > 30% trabecular/insular/solid architecture, tumor necrosis, and mitotic count of > 3 per 10 high-power fields. The first two of these exclusion criteria distinguish NIFTP from PTC; the latter three distinguish NIFTP from poorly differentiated thyroid carcinoma. Morphologic features of specific PTC variants, such as tall cells, columnar cells, and cribriform architecture would also exclude the use of NIFTP. For quantifying mitotic figures, the working group recommends evaluating 10 consecutive high-power fields, rather than preferentially examining areas of elevated activity [71]. Psammoma bodies represent calcified remnants of papillae, and are consequently inconsistent with NIFTP [72].

Subsequent study and evaluation seems to justify separation of these tumors into a new category, while providing information about the malignant potential of NIFTP [reviewed in 71]. A thorough review by the working group yielded approximately 600 cases that satisfied diagnostic criteria for NIFTP. Among these tumors, the reviewers identified up to three recurrences/distant metastases [57,68-70,73,74]. One recent study examined the effect of tightening the definition of NIFTP, to exclude cases containing a single papillary structure. Even with this stringent requirement, central lymph node micrometastasis still occurred in 3% of cases. In the same series, one case with ≤1% subtle papillae (i.e. fulfilling original criteria for NIFTP) developed a bone metastasis. This tumor also harbored a BRAF V600E mutation. When complete absence of papillae was required for diagnosis of NIFTP, no tumors harbored this mutation [74].

Consequently, the diagnostic criteria were amended to exclude these rare cases with more aggressive behavior. Current recommendations discourage diagnosis of NIFTP if even a single bona fide papilla is present, or if diffuse PTC nuclei are evident. In tumors with florid nuclear features of PTC (nuclear score 3), the entire tumor should be examined to exclude the presence of papillae. The working group currently recommends “if there is difficulty in interpreting the pattern of growth, then NIFTP should not be the diagnosis applied” [71].

Verification of noninvasive status requires rigid grossing protocol. The working group recommends submission and evaluation of the tumor capsule in its entirety. According to the Royal College of Pathologists in the United Kingdom, tumors that otherwise fulfill criteria for NIFTP should be diagnosed as noninvasive encapsulated FVPTC if the entire capsule is not examined [75]. The presence of tumor at the surgical margin also precludes diagnosis of NIFTP, as this scenario would prevent assessment of the tumor's entire periphery.

In addition to the follicular-patterned lesions discussed above, the differential diagnosis of NIFTP includes two other entities recognized by the WHO: well differentiated tumor of uncertain malignant potential (WDT-UMP), and follicular tumor of uncertain malignant potential (FT-UMP) [76]. These tumors are encapsulated or well-circumscribed, show follicular architecture, and questionable capsular or vascular invasion, with (WDT-UMP) or without (FT-UMP) PTC nuclear features. Atypical adenoma is another synonym for these lesions.

The Chernobyl Pathologists Group originally proposed the category of WDT-UMP [77]. Another group proposed the related term “well-differentiated tumor of uncertain behavior” to include WDT-UMP, encapsulated FVPTC, and other encapsulated follicular-patterned lesions (including minimally invasive FTC), with equivocal PTC nuclear features [78]. A related entity, FT-UMP, is distinct from WDT-UMP insofar at the former lacks nuclear features of PTC. This terminology was also proposed by the Chernobyl Pathologists Group, specifically for cases with questionable capsular invasion. The WHO currently includes cases with equivocal vascular invasion in this category as well.

Diagnostic language imparting “uncertain malignant potential” poses a quandary for surgeons and endocrinologists, regarding optimal

management of their patients. This terminology does not easily facilitate a decision between conservative (e.g. lobectomy) versus more aggressive management (e.g. completion thyroidectomy and post-operative radioactive iodine). Definitive placement of tumors within benign or malignant categories is preferred. In the absence of malignant diagnostic features, a conservative diagnostic approach is advocated, if necessary with a recommendation for close follow-up/monitoring, including periodic imaging studies and serum thyroglobulin levels.

The definition of and criteria for NIFTP will likely continue to evolve. Several scenarios require additional investigation and clarification. These issues include multifocal tumors, and lesions measuring < 1 cm in greatest dimension. Data regarding these situations is limited [73]. NIFTP terminology is currently permitted in these contexts, but more study is necessary.

Another unresolved question pertains to Hürthle cell lesions that satisfy criteria for NIFTP. Nuclear morphology in Hürthle cells may be challenging to evaluate, among other considerations. Although the Endocrine Pathology Society working group currently allows diagnosis of NIFTP for oncocytic lesions, the United Kingdom Royal College of Pathologists excludes the diagnosis in this scenario [75]. As above, further studies may be contributory.

6. Molecular findings: FA/FTC/FVPTC

Molecular alterations in thyroid tumors generally comprise two categories [reviewed in 79]. One set of mutations is associated with follicular-patterned tumors, including FA, FTC, and encapsulated FVPTC. The most common alterations in FTC are *RAS* point mutations and *PPARG* gene fusions. In most series, mutations in *NRAS*, *HRAS*, or *KRAS* have been reported in 33–56% of FTC, and 15–48% of FA [80-86]. The most commonly affected hotspot is codon 61 of *NRAS*, followed by codon 61 of *HRAS*, whereas *KRAS* is less frequently mutated. Rates of *RAS* mutation in FTC do not appear to differ between iodine-rich and iodine-deficient countries [87].

Other follicular-patterned tumors harbor *RAS* mutations as well. *NRAS*, *HRAS*, and *KRAS* mutations have been reported in NIFTP and FVPTC [70,82]. Namba et al. [81] and Soares et al. [84] report *RAS* mutations in 21% and 7% of PTC cases in their series, respectively; these authors do not specify whether the PTC cases were FVPTC. Interestingly, Nikiforova et al. [85] reported a *RAS* mutation in 1 of 19 classical PTC cases. *RAS* mutations have also been reported in 4–21% of multinodular goiters [81,82,85]. The presence of *RAS* mutations at all stages of thyroid neoplasia supports the concept that *RAS* mutation occurs early in tumorigenesis [80]. Unlike other follicular-patterned tumors, Hürthle cell tumors infrequently harbor *RAS* mutations [83,85].

PPARG (peroxisome proliferator-activated receptor γ1) fusions represent the other component of this molecular signature. Rearrangements involving *PPARG* occur either as *PAX8-PPARG*, or, less frequently, *CREB3L2-PPARG*. The former comprises the thyroid transcription factor PAX8, and domains A to F of PPARG, and results from t(2;3)(q13;p25) [88]. The latter consists of the transactivation domain of CREB3L2 and all functional domains of PPARG, and results from t(3;7)(p25;q34) [89]. *PPARG* fusions are seen primarily in FTC (11–57% in most series) [83,90-95]. *PAX8-PPARG* fusion may also be seen, albeit at lower frequencies, in FA (4–13%) and FVPTC [83,86,90,91,94]. In a single series, *PAX8-PPARG* rearrangement occurred more frequently in FA than FTC [86]. Investigations of its significance have shown that FTC harboring *PPARG* rearrangement tends to present at a younger patient age, and more frequently exhibits vascular invasion [83,91,92]. In one study, positive immunohistochemical staining for PPARG was associated with favorable prognosis [94]. Interestingly, one report describes FTC with both *RAS* mutation and *PAX8-PPARG* fusion [83]. As with *RAS* mutations, Hürthle cell tumors infrequently have *PPARG* rearrangements [83].

Other alterations occur in follicular-patterned tumors as well. A study of 29 lesions harboring the *BRAF* K601E mutation included one FA, one FTC, and 20 FVPTC (the remaining 7 cases were non-follicular variant PTC) [96]. Other authors have also identified K601E in FA [84], as well as NIFTP [reviewed in 71]. *PTEN* mutations have also been reported in hyperplastic nodules, 7% of FA, and 6–27% of FTC [85,97,98]. Other alterations in NIFTP within the *RAS*-like molecular spectrum include *THADA* fusions, and *EIF1AX* mutations.

Some mutations that occur in follicular-patterned tumors are characteristic of more aggressive entities. *TERT* promoter mutations, located either 124 or 146 base pairs upstream of the start codon, have been described as recurrent alterations in FTC (as well as PTC). However, they are present at higher frequency in widely invasive Hürthle cell carcinoma, poorly differentiated thyroid carcinoma, and anaplastic thyroid carcinoma (ATC). Furthermore, studies have shown *TERT* promoter mutations impart increased risk for disease-specific mortality, distant metastasis, and persistent disease [reviewed in 79].

PIK3CA mutations and copy-number gains are also found in FTC. Studies have shown *PIK3CA* mutations in 13% of FTC [98], and *PIK3CA* amplification (≥4 copies) in 8–12% of FA, and 24–29% of FTC [98,99]. Mutations of *PIK3CA* are associated with aggressive behavior and tumor progression, and are seen more frequently in poorly differentiated thyroid carcinoma and ATC [100]. Coexistence of *PIK3CA* pathway alterations is seen with progression from differentiated tumors to ATC. This observation conveys the importance of PI3K pathway activation in thyroid tumorigenesis, particularly in FTC and ATC. Aberrant pathway activity may promote progression from FA to FTC to ATC as alterations accumulate within this pathway [100].

Some molecular alterations predict benign behavior, such as activating mutations of *TSHR* and *GNAS* [reviewed in 79]. Approximately 57–80% of hyperfunctioning nodules harbor *TSHR* mutations, and approximately 3–6% of these nodules harbor *GNAS* mutations [85,101]. *TSHR* and *GNAS* mutations are found in most hyperfunctioning adenomata, and *TSHR* mutations may be seen in FTC, particularly in hyperfunctioning FTC [31, reviewed in 102].

7. Molecular findings: other tumors

Another set of molecular alterations is more characteristic of PTC. Much of this information comes from the TCGA study of PTC [103, reviewed in 79]. Mutations of *BRAF* are seen in approximately 40–46% of PTC [84]. Activation of BRAF signaling may also occur through fusion of *BRAF* with partners such as *AKAP9*, *SND1*, or *MKRN1*. Fusions involving *RET* are the other feature of this molecular signature. *RET-PTC1* (fusion of *RET* with *CCDC6*), and *RET-PTC3* (fusion of *RET* with *NCOA4*) are reported in 10–30% of PTC.

Other molecular alterations also occur in PTC [reviewed in 79]. Rearrangements involving *NTRK1* and *NTRK3* are seen in 5% of cases, and perhaps even more frequently among pediatric patients with PTC. Approximately 1% of cases harbor fusions involving *THADA* [103]. Like with FA/FTC, some alterations observed in PTC are more characteristic of aggressive entities. Fusions involving the *ALK* gene are present in approximately 1–2% of PTC, 4–9% of poorly differentiated thyroid carcinoma, 4% of ATC, and 1–2% of medullary thyroid carcinoma. *EIF1AX* is a translational initiation factor, and mutations of it occur throughout the entire spectrum of thyroid lesions (reported in one hyperplastic nodule, two cases of FA, 2% of PTC, 11% of poorly differentiated thyroid carcinoma, and 9% of ATC).

Molecular findings characteristic of PTC are considered inconsistent with a diagnosis of NIFTP. Notably, one recent study reports *BRAF* mutations in 28.6% of NIFTP cases [104]. As stated above, another series included a tumor that satisfied original criteria for NIFTP, although it harbored a BRAF V600E mutation, and the patient developed a bone metastasis [74]. These findings underscore the importance of additional investigation regarding the entity's molecular pathology, and the need to exclude tumors with PTC-type alterations.

Molecular findings in Hürthle cell neoplasia are unique, and justify

placement of these lesions in a separate diagnostic category. The metabolic and phenotypic alterations that distinguish these tumors from their non-Hürthle counterparts result from characteristic genetic alterations. These alterations include large deletions of mitochondrial DNA, and mutations of mitochondrial and nuclear genes, including the mitochondrial and nuclear genes for subunits of complex I (NADH coenzyme Q reductase). This complex represents one of five multimeric complexes that occupy the inner mitochondrial membrane, and constitute the oxidative phosphorylation system [105, reviewed in 106]. These mutations, and the consequent disruption of oxidative phosphorylation, cause deficits of energy production in the neoplastic cells. These deficits lead to compensatory accumulation of mitochondria. Accordingly, the genetic lesions of Hürthle cell tumors are thought to engender an aberrant mitochondrial compensatory mechanism, which accounts for the oncocytic phenotype.

Somatic and germline mutations affecting a complex subunit encoded by the *NDUFA13* nuclear gene (also called *GRIM19*) occur uniquely in oncocytic tumors. In one study, somatic missense mutations of *GRIM19* were detected in 3 of 20 sporadic Hürthle cell carcinomas. A germline mutation was also detected in an oncocytic PTC, arising in a thyroid with multiple Hürthle cell nodules [107].

Other studies investigating the molecular pathology of Hürthle cell tumors have shown mutational, transcriptional, and copy number profiles distinct from those of PTC and FTC, emphasizing the unique status of Hürthle cell carcinoma among thyroid malignancies [108]. Transcriptome signatures of these tumors are consistent with activation of the WNT/beta-catenin and PI3K/AKT/mTOR pathways. *TP53* mutations have been identified in up to 42% of Hürthle cell carcinomas, sometimes in association with *PTEN* mutations [109].

8. Molecular findings: NIFTP

Next-generation sequencing was performed using 37 NIFTP cases from the original (definitive) cohort, targeting point mutations and indels within 14 genes (*AKT1*, *BRAF*, *CTNNB1*, *EIF1AX*, *GNAS*, *HRAS*, *KRAS*, *NRAS*, *PIK3CA*, *PTEN*, *RET*, *TP53*, *TSHR*, and *TERT*) and 42 gene fusions involving *ALK*, *BRAF*, *NTRK1*, *NTRK3*, *PPARG*, *RET*, and *THADA* [110]. Almost 80% of NIFTP cases harbored clonal alterations. As above, the molecular profile of NIFTP resembles those of other follicular-patterned lesions, rather than PTC.

Molecular pathology has provided insights about the relationships between various follicular-patterned tumors. One particularly interesting concept relates to the molecular similarities between NIFTP and FA/FTC/encapsulated FVPTC, as opposed to classical PTC/infiltrative FVPTC. A paradigm emerges according to which NIFTP represents a precursor lesion of invasive encapsulated FVPTC, driven by *RAS/PPARG*-type alterations, whereas classical PTC and infiltrative FVPTC arise due to different molecular lesions [reviewed in 111]. Molecular features of NIFTP require ongoing investigation, which will likely enhance our understanding of the relationships between these tumors.

9. Conclusions

One review described follicular-patterned thyroid tumors as “the bane of the pathologist” [112]. Without comment regarding potential hyperbole by these expert authors, we concede the accuracy of their gist. Thyroid neoplasia with follicular architecture spans a considerable array of distinct yet similar entities, with very different prognostic and therapeutic implications. Placement of these lesions into the proper diagnostic category requires thorough gross techniques, careful attention to morphologic features and pathologic context, and (potentially) understanding of adjunct molecular findings.

The diagnostic approach to these tumors has changed significantly over many decades, beginning with the definition of FVPTC, and continuing most recently with the inception of NIFTP. Other changes have also occurred during this interval, such as the recognition that Hürthle cell tumors represent a unique category of follicular-patterned neoplasia. Ongoing investigation will facilitate enhanced definition of these lesions, and better understanding and management of this diverse group of tumors.

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