Capsule of Follicular Cell Tumors, its Significance and Morphology

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Capsule of thyroid nodule

NODULAR GOITER
FOLLICULAR ADENOMA
FOLLICULAR CARCINOMA
PAPILLARY CARCINOMA
POORLY DIFFERENTIATED CARCINOMA
MEDULLARY CARCINOMA
OTHERS

Capsule of thyroid nodule

Nodular goiter
Follicular adenoma/carcinoma

Capsule in papillary thyroid carcinoma

NON-ENCAPSULATED TYPE
ENCAPSULATED TYPE
CYSTIC TYPE

Macroscopic types of papillary thyroid carcinomas

- Encapsulated type: 6%
- Partly encapsulated type: 11%
- Non-encapsulated type: 75%
- Cystic type: 7%

(150 PTCs with classical histology)

Diagnosis of follicular variant of papillary thyroid carcinoma

PROBLEMATIC ISSUE

**Encapsulated Follicular Variant of Papillary Thyroid Carcinoma**

- Tumours with a small number of PTC nuclei and/or questionable PTC nuclei often diagnosed
- High observer variation
- WITH Invasion
- WITHOUT Invasion

**EFV-PTC FTC**

- WDT-IMP: WELL-DIFFERENTIATED TUMOUR OF INDETERMINED MALIGNANT POTENTIAL

This tumor should be treated as **BENIGN**.

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**Follicular Adenoma/Carcinoma**

**Adenoma**
- A BENIGN ENCAPSULATED TUMOUR OF THE THYROID SHOWING EVIDENCE OF FOLLICULAR CELL DIFFERENTIATION

**Carcinoma**
- A MALIGNANT EPITHELIAL TUMOUR SHOWING EVIDENCE OF FOLLICULAR CELL DIFFERENTIATION AND LACKING THE DIAGNOSTIC NUCLEAR FEATURE OF PAPILLARY CARCINOMA.

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**Diagnostic algorithm of follicle forming thyroid nodule**

- Papillary carcinoma cell nuclei
- Thyroid nodule
- Invasion

**Papillary carcinoma**
- Hyalinizing trabecular tumour

**Nodular goiter**
- Follicular a./c.
- Poorly diff. carcinoma

**Follicular carcinoma**
- Poorly diff. carcinoma
- Solid, trabecular, insular pattern, necrosis, mitosis

**Follicular a.**
- Nodular goiter

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**Perhaps the Most Difficult Problem in Thyroid Pathology is the Distinction of Follicular Carcinoma from Follicular Adenoma and from the Follicular Variant of Papillary Carcinoma.**

Evans HL. Cancer 54:535-540, 1984
**Invasions**

- Capsular invasion
- Vascular invasion

**My Procedure**

- Cover glass 24mm
- Longitudinal sections
- Nodule ≤ 2cm: Entire nodule
- Nodule > 2cm: 10 sections + Additional sections
- Nodulesctomy, lobectomy, subtotal/total thyroidectomy

**Invasion**

1. **Capsular Invasion (CI)**

Patterns of Capsular Invasion (CI)

Tumour penetration through the tumour capsule

Capsular invasion is defined by tumour penetration through the tumour capsule unassociated with the site of a previous fine needle aspiration biopsy. (WHO 2004)

Patterns of Capsular Pseudoinvasion (CPI)

Tumour penetration at the tumour capsule

Capsular pseudoinvasion is characterized by tumour penetration through the tumour capsule at the site of a previous fine needle aspiration biopsy.
WHAFFT
Worrisome histologic alterations following FNA of the thyroid
LiVolsi VA, Merino MJ. Lab Invest 62, 1990

- Hemorrhage and hemosiderin
- Focal and geographic pattern of lesion

2. VASCULAR INVASION (VI)

Patterns of Vascular Invasion (VI)
Capsular invasion is defined by the presence of intravascular tumour cells either covered by endothelium or associated with thrombus.
(WHO 2004)

Patterns of Pseudovascular Invasion (VI)
Artificially dislodged tumour cell (or tissue)

DIAGNOSIS OF FOLLICULAR CARCINOMA
Follicular tumours

- Showing definite invasion:
  - Follicular carcinoma
  - Follicular tumours of uncertain malignant potential (FT-UMP)

- Showing questionable invasion:
  - PTC nuclei (-)
  - PTC nuclei (questionable)
  - Well differentiated tumour of uncertain malignant potential (WDT-UMP)

**Immunohistochemical Demonstration of Lymphatic and Blood Vessels**

- Elastic tissue stains: only limited use in identifying intracapsular vessels

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**Immunohistochemistry**

<table>
<thead>
<tr>
<th>Vascular Markers</th>
<th>Lymphatic Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor VIII, CD31/34</td>
<td>FLT4, Live-1, D2-40</td>
</tr>
<tr>
<td>Laminin, Type IV collagen</td>
<td>D2-40</td>
</tr>
<tr>
<td>Ulex europaeus lectin</td>
<td></td>
</tr>
</tbody>
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**Distribution of Lymphatic and Blood Vessels in the Follicular Thyroid Tumours**

- Intratumorallymphatic vessel density was significantly higher in the follicular variant of PTC than in either follicular adenoma or follicular carcinoma.

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**Distribution of Lymphatic Vessels in Encapsulated Follicular Tumors**

- Intracapsular lymphatic vessels: scant or none

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Intratumoral lymphatic vessel density was significantly higher in the follicular variant of PTC than in either follicular adenoma or follicular carcinoma.
**Distribution of Blood Vessels in Encapsulated Follicular Tumors**

- CD34 staining
- Blood vessels

**Distribution of Lymphatic and Blood Vessels**

- CD34 staining for blood vessels (○)
- D2-40 staining for lymphatic vessels (○)

Follicular carcinomas tend to give rise to hematogenous spread and do not usually show lymphatic spread possibly due to lymphatic and blood vessel distributions.

**Thickness of Tumor Capsule**

The capsule of follicular carcinoma tends to be thicker and more irregular than that of adenoma.

- Evans, H.L. Cancer 54:535-540, 1984

**Capsule Thickness**

- Follicular adenomas (20 cases)
- Minimally invasive follicular thyroid carcinomas (48 cases)

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of cases</th>
<th>Capsule thickness (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular adenoma</td>
<td>20</td>
<td>mean: 174, SD: 30.9</td>
</tr>
<tr>
<td>Follicular carcinoma</td>
<td>48</td>
<td>mean: 407, SD: 33.8</td>
</tr>
</tbody>
</table>

The follicular carcinoma tends to be surrounded by a wide thick capsule.

**Subtypes of FTC and Recurrence and/or Metastasis**

- Minimally invasive FTC
- Widely invasive FTC

<table>
<thead>
<tr>
<th>Subtype</th>
<th>No. of cases</th>
<th>No. of cases with recurrence and/or distant metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimally invasive FTC</td>
<td>48</td>
<td>11 (23%)</td>
</tr>
<tr>
<td>Widely invasive FTC</td>
<td>14</td>
<td>11 (79%)</td>
</tr>
</tbody>
</table>

**No. of Vascular Invasion in Subtypes of FTC**

- Minimally invasive FTC
- Widely invasive FTC

<table>
<thead>
<tr>
<th>Subtype</th>
<th>No. of vascular invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimally invasive FTC</td>
<td></td>
</tr>
<tr>
<td>Widely invasive FTC (INC. POORLY)</td>
<td></td>
</tr>
</tbody>
</table>

No. of vascular invasion:

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>No. of vascular invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimally invasive FTC</td>
<td>48</td>
</tr>
<tr>
<td>Widely invasive FTC</td>
<td>14</td>
</tr>
</tbody>
</table>

- More than 4
- Capsule invasion alone
**NO. OF VASCULAR INVASION AND RECURRENCE AND/OR METASTASIS IN MINIMALLY INVASIVE FTC**

<table>
<thead>
<tr>
<th>No. of vascular invasion (VI)</th>
<th>No of case</th>
<th>No of vascular invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI&lt; 4</td>
<td>37</td>
<td>15 (23%)</td>
</tr>
<tr>
<td>VI&gt;= 4</td>
<td>11</td>
<td>2 (18%)</td>
</tr>
</tbody>
</table>

**SIGNIFICANCE OF INVASION**

- Follicular carcinoma
  - Encapsulated
    - With capsular invasion only
    - With limited (<4) vascular invasion
    - With extensive (>=4) vascular invasion
  - Widely invasive

There is a prognostic difference depending on the number of vessels involved.

**STI PATTERN IN MINIMALLY INVASIVE FTC**

<table>
<thead>
<tr>
<th>STI pattern</th>
<th>No of case</th>
<th>Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>15</td>
<td>5 (33%)</td>
</tr>
<tr>
<td>Present</td>
<td>33</td>
<td>6 (18%)</td>
</tr>
</tbody>
</table>

**DISTANT METASTASIS IN MINIMALLY INVASIVE FTC**

- Tumour size: Not significant for metastasis
- Gender: Not significant for metastasis
- Capsule thickness: The capsule of MI-FTC is thicker than that of adenoma
- Capsular invasion: Not significant for metastasis
- Vascular invasion: Number of VI could be important
  - With extensive (>=4) vascular invasion
- Solid, Trabecular, and Insular patterns: Possible significant marker
ENCAPSULATED MALIGNANT FOLLICULAR CELL DERIVED THYROID TUMORS

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Introduction:

Despite past and recent efforts, there are many problems and controversies in the classification of thyroid carcinomas of follicular cell origin. These controversies have an impact on the prognosis and therapy of thyroid carcinomas patients as well as on the development of robust cutting edge research aimed at better outcome and quality of life. The most controversial class of carcinomas is encapsulated malignant follicular cell derived tumors. This group of carcinoma include frequently encountered tumors such as the follicular variant of papillary thyroid carcinomas and encapsulated (so called “minimally invasive”) follicular carcinomas as well as rare neoplasms such as encapsulated poorly differentiated and anaplastic carcinomas. This lecture will focus on the two most common and problematic entities: the follicular variant of papillary thyroid carcinoma and encapsulated “minimally invasive” follicular carcinoma.

Follicular variant of papillary thyroid carcinoma:

The follicular variant of papillary thyroid carcinoma (FVPTC) is the most common subset of papillary carcinoma and is found in 9% to 22.5% of patients with papillary carcinoma (PTC) (1-4). This variant is composed entirely or almost completely of follicles, which are lined by cells having the nuclear features of papillary carcinoma (5). Thus, FVPTC shares with follicular adenoma (FTA) and carcinoma (FTC) the presence of follicles. When FVPTC is nonencapsulated and infiltrates the surrounding thyroid parenchyma or diffusely involves the thyroid, the diagnosis of carcinoma usually poses no problem. For the encapsulated tumor without invasion of surrounding thyroid tissue, the diagnosis of malignancy relies solely on the presence of the nuclear features of PTC.
(e.g., nuclear clearing, overlapping, grooves, pseudoinclusions), which often can be borderline. Therefore, the diagnosis of noninvasive, encapsulated FVPTC versus follicular adenoma is prone to considerable interobserver variability (6,7). This diagnostic dilemma has very important therapeutic implications. Indeed, if an FVPTC measures ≥1.5 cm, then many physicians in the United States will recommend completion thyroidectomy followed by radioactive iodine therapy (RAI) (8). Some authors have suggested that patients with encapsulated, noninvasive FVPTC have an excellent prognosis and, thus, believe that only a lobectomy is needed (5). However, there are no outcome data with long median follow-up from a large number of patients with FVPTC. More important, to our knowledge, there has been no study in which tumor behavior was analyzed according the histologic “subvariants” of FVPTC. (i.e., nonencapsulated [infiltrative/diffuse] vs. encapsulated) that can serve as the basis for a conservative treatment approach for encapsulated, noninvasive FVPTC. In addition, there is some controversy regarding the classification of FVPTC as a member of the PTC group versus the FTA/FTC group. Indeed, Baloch and LiVolsi showed that some encapsulated FVPTCs metastasize to distant sites in the absence of lymph node metastases, mimicking the behavior of FTC (9). Other authors reported that FVPTC has a significantly lower metastatic lymph node rate and more often is encapsulated than classic PTC (10-11). Recently, several groups have attempted to analyze FVPTC at the molecular and chromosomal levels (10,12,13). All of those studies concurred that the molecular profile of the FVPTC seems to be closer to the FTA/FTC group than to classic PTC, supporting further consideration of the classification of FVPTC.
To assess the behavior of FVPTC (especially its encapsulated form) and to shed more light on its true position in the classification scheme of well differentiated thyroid carcinoma, we undertook a clinicopathologic study of all patients with FVPTC who were seen at Memorial Sloan-Kettering Cancer Center between 1980 and 1995 (14). FVPTC tumors were classified according to histologic growth patterns as encapsulated versus nonencapsulated (infiltrative/diffuse) neoplasms. The encapsulated subset was subdivided further according to the presence or absence of invasion in a manner similar to that used to differentiate FTA from FTC. The encapsulated FVPTCs outnumbered their infiltrative/diffuse counterparts (only 17 tumors were diffuse or infiltrative vs. 61 encapsulated FVPTCs). This rarity of infiltrative/diffuse (i.e., nonencapsulated) FVPTC seems to be concordant with the first detailed article on FVPTCs by Chem and Rosai (15).

In our study, patients who had infiltrative/diffuse FVPTC had a significantly greater frequency ($P < .0001$) of marked intratumoral fibrosis, extrathyroid extension, and positive margins than patients who had encapsulated FVPTC (15). This superior potential of nonencapsulated FVPTCs in invading the thyroid and extrathyroid stroma was reflected by its higher rate of total thyroidectomy and especially by its rate of regional lymph node metastases. Indeed, patients with nonencapsulated (infiltrative/diffuse) FVPTCs had a metastatic lymph node rate of 65% compared with 5% for patients with encapsulated FVPTCs ($P < .0001$). This difference in lymph node disease could not be explained by differences in tumor size, gender or age at presentation, because the latter two variables were similar between the patients in the encapsulated group and the nonencapsulated group. In our study, the metastatic lymph node rate of encapsulated
FVPTCs (5%) was much closer to that reported in follicular carcinomas (on the order of 5-10%), whereas infiltrative/diffuse FVPTCs had a metastatic lymph node rate within the range reported for classic papillary carcinomas (on the order of 45-65%). Our overall metastatic lymph node rate in FVPTCs (18%) was slightly higher than that reported by Zhu et al (13%) but lower than the frequency of lymph node disease reported by Zidan et al. and Tielens et al. (22%) (3,10, 16). The latter difference may be explained by the observation that those investigators allowed up to 20% of papillae in their FVPTCs (3). Their tumors will be now classified as classic PTC by most authors, because the modern and now universally accepted description of FVPTC indicates that the tumor must be entirely or almost completely follicular in pattern (17).

With regard to prognosis, patients who had invasive tumors, whether encapsulated or not, had a rare but real potential for adverse outcome (14). One patient who had an encapsulated FVPTC with capsular and vascular invasion developed a recurrence in the cervical lymph nodes 2 years after surgery. With a median follow-up of almost 11 years, overall, 6% of patients who had invasive tumors, whether encapsulated or not, had adverse outcomes, whereas none of the 42 patients who had noninvasive, encapsulated FVPTCs developed recurrences/metastases, or died of disease. All 31 patients who had noninvasive, encapsulated FVPTC and underwent lobectomy alone had good outcomes and no lymph node metastases (median follow-up, 11.1 years, median tumor size of 2.3 cm). These data confirm the view that patients with noninvasive, encapsulated FVPTC have an excellent prognosis (5). Eight of the noninvasive, encapsulated FVPTCs had multifocal distribution of the nuclear features of papillary carcinoma. We classified the entire tumor as FVPTC according to the recommendation of Baloch and LiVolsi
with the understanding that some pathologists may count the foci with atypical nuclei and report the lesion as multifocal PTC (9). Others may use alternative terminology, such as “tumor as of uncertain malignant potential” (19). Whatever position the investigator takes regarding the nuclear features of FVPTC, the current results seem to point to the importance of invasion rather than nuclear features for predicting outcomes in patients who have encapsulated FVPTC. Indeed, encapsulated FVPTC seems to have a behavior much closer to that of follicular tumors (i.e., FTA and FTC) rather than classic PTC. This is reflected by the lack of adverse outcomes in patients with noninvasive lesions and the rarity of lymph node metastases. These morphologic and clinical data are supported, as mentioned earlier, by several publications pointing to a molecular profile of FVPTC much closer to the FTA/FTC group than to classic PTC.

Indeed, Zhu et al have shown a low frequency of RET/PTC rearrangement (3%) and a high frequency of RAS mutations in FVPTC (43%), very similar to follicular carcinoma (10). Adeniran et al confirmed these findings and showed that within PTC, RAS mutations occur only in FVPTC and are associated with a low level of lymph node metastases (19). Moreover, BRAF mutations were found to be absent in FVPTC and FTC while present in 53-56% of classical PTC (19,20). When we genotyped FVPTC according to its encapsulated and infiltrative form, we found that encapsulated FVPTC have a molecular profile similar to follicular adenoma/carcinoma harboring RAS mutations in 36% of cases and no BRAF or RET/PTC alteration (21). In contrast, infiltrative FVPTC had a somewhat opposite profile displaying BRAF V600E mutations in 26% of the tumors while RAS mutations and RET/PTC rearrangement were present
each in 10% of the cases. The molecular profile of infiltrative FVPTC is in between FTA/FTC and classical PTC albeit closer to the latter.

The histopathologic, clinical and molecular data gathered to date strongly argues for FVPTC being two diseases: 1) Encapsulated FVPTC with a genotypic, invasive and behavioral profile similar to follicular adenoma/carcinoma 2) Infiltrative FVPTC with invasive and behavioral features very close to classical PTC and a molecular profile closer to classical PTC than to FTA/FTC. The total lack of recurrence in our group of patients with noninvasive FVPTC who underwent lobectomy alone suggests that noninvasive, encapsulated FVPTC may be managed by lobectomy only, as recommended by Rosai et al. in the last Armed Forces Institute of Pathology fascicle on thyroid tumors (5). Larger studies with longer follow-up than the current series will be needed to refine therapy for patients with noninvasive, encapsulated FVPTC. If the current findings are confirmed, then strong consideration should be given to reclassifying encapsulated FVPTC as an entity that is close to the FTA/FTC class of tumors. The same criteria that were used to decide whether follicular tumors are biologically benign or malignant (i.e., capsular and vascular invasion) would be applied to the evaluation of encapsulated FVPTCs. In practical terms, lack of capsular or vascular invasion should denote a benign clinical behavior in encapsulated FVPTC. If this reclassification is realized, then it will have a major impact on the diagnosis and management of patients with FVPTC. In noninvasive, encapsulated FVPTC, pathologists will be spared the frustrating and subjective exercise of deciding whether a tumor has the nuclear features of papillary carcinoma. More importantly, countless numbers of patients with noninvasive,
Encapsulated FVPTC will be spared unnecessary and aggressive therapy with its attached morbidity (i.e., hypoparathyroidism and recurrent nerve injury) and financial costs.

**Extent of invasion in Follicular carcinoma (minimally versus widely invasive):**

Follicular carcinomas of the thyroid gland, including its oncocytic variant (so-called Hurthle cell carcinoma), are subdivided into minimally invasive and widely invasive tumors (5). The minimally invasive carcinoma, also termed encapsulated, is totally surrounded by a fibrous capsule and displays capsular invasion and/or foci of vascular invasion. It is unusual for these foci of invasion to be detected grossly (5). In contrast, widely invasive follicular and Hurthle cell carcinoma are defined by extensive areas of invasion at both the macroscopic and microscopic level (5). This classification correlates very well with outcome, such that minimally invasive tumors have an overall excellent prognosis, whereas widely invasive tumors have a much poorer outcome (5). However, there are cases of encapsulated minimally invasive follicular carcinoma that recur and metastasize (22,23). Identifying these cases at the time of diagnosis is crucial because a minimally invasive tumor will be treated by lobectomy alone followed by observation in some centers, even if the follicular tumor is of the oncocytic (Hurthle cell) category. This approach will risk undertreating those few minimally invasive tumors with a poor outcome. At variance with this minimalist surgical approach, many surgeons will perform a total thyroidectomy for any minimally invasive follicular carcinoma, especially those of the oncocytic category, most likely overtreating a large number of cases. In order to detect those “minimally invasive” cases that recur, some authorities limit the use of the minimally invasive term to those carcinomas with capsular invasion only since their
metastatic rate is close to 0 (24,25). The designation “grossly encapsulated angioinvasive follicular carcinoma” has been suggested to encapsulated tumors with any foci of vascular invasion in view of their perceived higher risk of recurrence (26). These authors feel that the presence of vascular invasion, even in one or a few endothelial-lined vessels, negates a diagnosis of “minimal invasion” as these carcinomas once gaining access to any vessel have the capacity to behave in a more aggressive manner than those carcinomas with only capsular invasion (26). Other authors base their definition of minimally invasive carcinoma on the number of foci of invasion especially vascular invasion. (25, 27-29). We attempted to identify prognostic factors of recurrence in a series of 50 encapsulated follicular carcinomas, oncocytic variant (Hurthle cell) treated at Memorial Sloan-Kettering Cancer Center (28). Forty seven percent of these encapsulated Hurthle cell carcinomas with 4 or more foci of angioinvasion recurred. (28). Extensive capsular invasion, gender, and age did not confer a statistically higher recurrence rate. This correlation between extent of vascular invasion and adverse outcome was found in a small study of encapsulated non-Hürthle cell follicular carcinomas (29) and in larger studies analyzing follicular carcinomas in general without emphasis on its oncocytic variant. (25-30). It is truly remarkable that the number of foci of vascular invasion we determined to place patients at high risk of disease recurrence (≥4 vessels) is very close to the number found by Lang et al in 1986 (>4 foci) (25). Based on the above data, we label tumors that have 4 or more foci of vascular invasion as “grossly encapsulated follicular carcinoma with extensive angioinvasion”. In another study, we showed that follicular oncocytic (Hürthle cell) carcinomas with a total of 2 foci of capsular/vascular
invasion did not recur after a long follow up and should therefore be called minimally invasive (27).

Irrespective of one’s philosophy in regard to the definition of minimally invasive follicular carcinoma, pathologists should report on the presence as well as the extent (focal, extensive) of capsular and lymph-vascular invasion. This approach has a dual advantage of collating the various terminologies suggested for these carcinomas, as well as and perhaps more importantly, providing a report that better assists the clinician in assessing recurrence risk and, therefore, in deciding on the extent of surgical intervention (e.g., completion thyroidectomy) and the use of postoperative RAI therapy.

**Conclusion:**

Thyroid carcinomas of follicular cell origin, especially encapsulated carcinomas are in need of continuous reclassification. The prognosis and therapy of many entities can be better delineated if a meticulous microscopic examination is performed. An accurate assessment of the extent of invasion (especially vascular) is crucial. In addition, molecular data gathered in the last 20 years can help reassess these tumors at the morphological level into clinically relevant histopathologic entities, as in the case of the FVPTC.
References:


"New Insight in the Encapsulated Thyroid Follicular Cell Tumors"

2010 Endocrine Pathology Companion Meeting
Washington, DC

“Familial Follicular Cell Tumors: Classification and Morphological Characteristics"

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Handout for the USCAP Endocrine Pathology Companion Meeting, Washington, DC, March 2010
**Introduction:**

Although the majority of thyroid carcinomas are sporadic, familial cases represent from 5% to over 25% of cases. Familial syndromes are classified into familial medullary thyroid carcinoma, and familial tumors derived from follicular cells, also called non-medullary thyroid carcinoma.

Although, the majority of follicular cell-derived papillary and follicular thyroid carcinomas are sporadic, the familial forms of follicular-derived neoplasms have only been acknowledged in recent years. The familial forms of follicular-derived tumors are rare and encompass a heterogeneous group of diseases, including both syndromic-associated tumors, and non-syndromic tumors (1, 2).

The syndromic-associated group has an increased prevalence of follicular cell-derived tumors within a familial cancer syndrome with a preponderance of non-thyroidal tumors - familial tumor syndromes characterized by a preponderance of non-thyroidal tumors. Thyroid neoplasia has been reported with increased frequency in familial syndromes, such as familial adenomatous polyposis (FAP), *PTEN* hamartoma tumor syndrome (PHTS), Carney complex type 1, and Werner syndrome.

In the second group, non-syndromic tumors, the predominant neoplasm is non-medullary thyroid carcinoma (familial tumor syndromes characterized by a predominance of follicular cell derived tumors), although other neoplasms may occur with increased frequency. This second group also includes pure familial PTC with or without oxyphilia, fPTC with papillary renal cell carcinoma, and fPTC with multinodular goiter. Familial follicular cell-derived tumors or non-medullary thyroid carcinoma are characterized by three or more first degree relatives with follicular-derived non-medullary thyroid carcinoma, and occurs regardless of the presence of another familial syndrome.
Familial Follicular Cell Tumors: Classification

The familial follicular cell-derived tumors or familial non-medullary thyroid carcinomas (FNMT) encompass a heterogeneous group of diseases, including both syndromic-associated tumors, and non-syndromic tumors (1, 2). We will describe the most common syndromes associated with follicular cell tumors.

1 - Familial Tumor Syndromes Characterized by a Preponderance of Non-Thyroidal Tumors:

\textit{PTEN}-Hamartoma Tumor Syndrome (PHTS):

Cowden Syndrome (CS) and Bannayan-Riley-Ruvalcaba Syndrome (BRRS) are the major entities comprising the \textit{PTEN} Hamartoma Tumor Syndrome (PHTS), which is caused by germline mutations of the \textit{PTEN} gene and inherited in an autosomal dominant fashion. \textit{PTEN} (Phosphatase and Tensin homolog deleted on chromosome Ten) is a tumor suppressor gene located on 10q23.3. Over 90% of individuals affected with CS manifest a phenotype by the age of 20 years. By the end or during their third decade almost all patients (99%) develop at least the pathognomonic mucocutaneous lesions. Thyroid pathologic findings in this syndrome typically affect follicular cells (3, 4). Affected individuals with CS develop both benign and malignant tumors in a variety of tissues, such as breast, uterus, and thyroid. Two thirds of CS patients develop thyroid tumors; the pathologic findings in this syndrome have been described as involving the follicular cells, and include multinodular goiter, multiple adenomatous nodules, follicular adenoma, follicular carcinoma, and less frequently, papillary thyroid carcinoma (3-6)

Familial Adenomatous Polyposis (FAP):

Familial adenomatous polyposis (FAP) is an inherited autosomal dominant syndrome caused by germline mutations in the adenomatous polyposis coli (\textit{APC}) gene on chromosome 5q21, characterized by hundreds of adenomatous colonic polyps that develop during early
adulthood. Extracolonic manifestations in FAP include osteomas, epidermal cysts, desmoid tumors, upper gastrointestinal tract polyps-hamartomas, congenital hypertrophy of the retinal pigmented epithelium (CHRPE), hepatoblastomas and thyroid tumors. Papillary thyroid carcinoma is one of the extracolonic manifestations of FAP, and occurs in approximately 2% of patients. Young women with FAP are at particular risk of developing thyroid cancer, and their chance of being affected is approximately 160 times greater than that of normal individuals; PTC occurs with a frequency of about 10 times greater than that expected for sporadic PTC (7-9).

2 - Familial Tumor Syndromes Characterized by a Predominance of Non-Medullary Thyroid Carcinoma:

Familial follicular cell-derived thyroid carcinoma syndrome or non-medullary thyroid carcinoma is diagnosed when three or more family members have nonmedullary thyroid cancer in the absence of other known associated syndromes.

Familial non-medullary thyroid carcinoma is rare and now recognized as a distinct clinical entity. FNMTT patients have shorter disease-free survival than do sporadic disease patients because of frequent locoregional recurrence (10-13).

The genetic inheritance of FNMTT remains unknown, but it is believed to be an autosomal dominant mode with incomplete penetrance and variable expressivity. Genetic analyses of large FNMTT kindreds represent the first steps in identification of the putative susceptibility genes (14-18).

**Familial Papillary Thyroid Carcinoma (fPTC)** is characterized by multicentric tumors, and multiple adenomatous nodules with or without oxyphilia (chromosomal region 19p13).

**The Familial Nonmedullary Thyroid Carcinoma Type 1 (fNMTC1) Syndrome** (chromosomal region 2q21) is characterized by PTC without any distinguishing pathologic features.
Familial PTC associated with papillary renal neoplasia syndrome (fPTC/ PRN), mapped to chromosomal region 1q21, includes not only PTC and the expected benign thyroid nodules, but also papillary renal neoplasia.

Familial Multinodular Goiter (FMNG) Syndrome (mapped to 14q), some patients may develop an associated PTC.
Familial Follicular Cell Tumors: Morphological Characteristics

1 - Familial Tumor Syndromes Characterized by a Preponderance of Non-Thyroidal Tumors:

**PTEN-Hamartoma Tumor Syndrome (PHTS):**

The majority of thyroid lesions occurring in PHTS are of follicular origin; characteristic multicentric, bilateral, benign and malignant thyroid lesions are observed in PHTS. Multiple adenomatous nodules (MAN) are a characteristic finding in PHTS, with multiple, up to 100 distinct well-circumscribed nodules, with a firm yellow-tan cut surface, diffusely involving the thyroid gland. In our experience, the adenomatous nodules seen in PHTS are bilateral, more numerous, and distinct from age-related nodules with only a scant atrophic rim of normal thyroid follicles. The number of these nodules far exceeds those seen in old age, or in any other inherited syndrome.

Follicular adenomas are very common, occur at earlier age, and usually are multicentric. Follicular carcinoma is a major criteria and an important feature in PTEN hamartoma tumor syndrome; these tumors are more frequently multicentric, and progress from a pre-existing follicular adenoma. However, PTC has rarely been associated with this entity.

Multiple adenomatous nodules in a background of lymphocytic thyroiditis and/or C-cell hyperplasia are distinctive findings in this syndrome, and should alert pathologists to notify clinicians of the possibility of PTEN Hamartoma Tumor Syndrome (1, 2).

**Familial Adenomatous Polyposis (FAP):**

Thyroid carcinomas associated with FAP are usually bilateral, and multifocal. The histological features are different from sporadic tumors with the characteristic cribriform pattern having solid areas and a spindle cell component, most often associated with marked fibrosis. The cribriform-morular variant of thyroid carcinoma (CMv-TC) typically occurs as an extraintestinal manifestation of familial adenomatous polyposis (FAP), initially described
as FAP-associated thyroid carcinoma (7), now classified as a variant of papillary thyroid carcinoma (9). The CMv-PTC is a very rare subtype of papillary thyroid carcinoma representing approximately 0.1-0.2% of all papillary carcinoma cases. Among patients with FAP who have synchronous PTC, over 90% of these cases have been reported to exhibit histologic features of the cribriform-morular variant.

The characteristic and unusual morphology is associated with follicular, papillary, trabecula, solid, spindle cell and squamoid areas with or without extensive fibrosis. The characteristic cellular and nuclear findings of sporadic PTC, such as nuclear grooving, overlapping, intranuclear inclusions, and clear nuclei are rare to absent in this subtype (7-9). This tumor may be encapsulated, have large bands of fibrosis and hyalinization, and have only scattered tumor cells embedded within fibrous tissue.

Immunostains of CMv-PTC show positivity for ER and PR, bcl-2, E-cadherin, and galactin-3. CMv-PTC is characterized by aberrant nuclear and cytoplasmic expression of beta catenin; immunostaining is strong in cytoplasm and nuclei in the morular and cribriform areas.

2. Familial Tumor Syndromes Characterized by a Predominance of Non-Medullary Thyroid Carcinoma:

Familial non-medullary thyroid carcinoma syndrome (FNMT) has been shown to be associated with the presence of multiple benign nodules, has a high incidence of multifocality, to behave in a more aggressive clinical behavior, and to have a worse prognosis than sporadic nonmedullary thyroid cancer. Individuals with FNMT have an increased risk of multifocal disease, local invasion, and increased local or regional recurrence and lymph node metastases. Compared to the patients with sporadic disease, the FNMT patients were more likely to have intraglandular dissemination. FNMT is an independent predictor of shorter disease-free survival (19, 20).

These tumors have no distinct morphological findings to differentiate them from the sporadic counterparts.

The pathologist is an important link in identifying these inherited tumors, and may play a crucial part in a patient’s care. Improved awareness and screening of FNMT will permit
earlier detection, a timely intervention, and improved outcomes for patients and their families.
References:


Introduction

Follicular cell thyroid tumors represent a challenging diagnostic area. Neoplastic follicular cells exhibit a wide degree of morphologic heterogeneity resulting in many growth patterns, i.e., follicular, papillary, solid, trabecular and insular, to name the most common. In addition, many tumors display admixtures of these growth patterns. The fundamental reasons for this morphologic heterogeneity are not entirely clear. However, we do know from numerous studies that the morphology of follicular cell thyroid tumors generally reflects their underlying genetic composition. Yet, even with the availability of genetic information, the narrative is somewhat cloudy. As was discussed in a recent editorial by Dr. Juan Rosai (1), different diagnoses can be derived for follicular cell thyroid tumors depending on the classification approach used (i.e., morphology, immunohistochemical profile, genotypic profile, and gene expression profile). Dr Rosai concluded it was time to go “Back to the Drawing Board” for the classification of some of these tumors, such as the encapsulated follicular variant of papillary carcinoma.

This presentation will present selected genetic research on encapsulated follicular cell tumors, with an emphasis on genotyping and genome-wide studies.

Genotyping Studies

By far, most of the genetic studies of follicular cell thyroid tumors have focused on defining mutations of specific genes known to be involved in the pathogenesis of these tumors and then examined their significance. As a consequence, much is known about a handful of genes in which mutations are thought to be dominant events in the initial development of tumors, so called driver mutations. (We now know that all mutations are not functionally equivalent. Thus, mutations are divided into two types: driver mutations and passenger mutations. See appendix). Driver mutations for follicular cell thyroid tumors include RET and NTRK1 rearrangements, RAS family point mutations, BRAF point mutations and rare rearrangements, PTEN mutations and likely PAX8/PPARY rearrangements. Mutations of CTNNB1 and TP53 are thought to be secondary mutations associated with histological progression to poorly differentiated and undifferentiated carcinoma. The Table below from Kondo et al. (2) summarizes the results from many genotyping studies.

<table>
<thead>
<tr>
<th>Genetic alteration</th>
<th>Well-differentiated thyroid carcinoma</th>
<th>Poorly differentiated thyroid carcinoma</th>
<th>Undifferentiated thyroid carcinoma</th>
<th>Post-Chernobyl childhood thyroid cancer</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>RET rearrangement</td>
<td>13–43%</td>
<td>0%</td>
<td>0–13%</td>
<td>0%</td>
<td>50–90%</td>
</tr>
<tr>
<td>BRAF mutation</td>
<td>29–69%</td>
<td>0%</td>
<td>0–13%</td>
<td>10–35%</td>
<td>0–12%</td>
</tr>
<tr>
<td>BRAF rearrangement</td>
<td>1%</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>11%</td>
</tr>
<tr>
<td>NTRK1 rearrangement</td>
<td>5–13%</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>3%</td>
</tr>
<tr>
<td>Ras mutation</td>
<td>0–21%</td>
<td>40–53%</td>
<td>18–27%</td>
<td>20–60%</td>
<td>0%</td>
</tr>
<tr>
<td>PPARG rearrangement</td>
<td>0%</td>
<td>25–63%</td>
<td>0%</td>
<td>0%</td>
<td>Unknown</td>
</tr>
<tr>
<td>CTNNB1 mutation</td>
<td>0%</td>
<td>0%</td>
<td>0–25%</td>
<td>66%</td>
<td>Unknown</td>
</tr>
<tr>
<td>TP53 mutation</td>
<td>0–5%</td>
<td>0–9%</td>
<td>17–38%</td>
<td>67–88%</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

CTNNB1, β-catenin; NTRK1, neurotrophic tyrosine kinase receptor, type 1; PPARG, peroxisome-proliferator-activated-receptor-γ.
Some conclusions from these studies regarding driver mutations in follicular cell thyroid tumors include the following:

- Most classical type papillary thyroid carcinomas (PTC) contain \textit{BRAF} mutations, unless radiation is involved, in which \textit{RET} rearrangements become more common.
- Most tall cell variants of PTCs contain \textit{BRAF} mutations.
- Most follicular variants of PTC contain \textit{RAS} mutations.
- Most follicular thyroid carcinomas (FTC) contain either \textit{RAS} mutations or \textit{PAX8/PPARγ} rearrangements.

Thus, it is quite apparent that there is a strong correlation between tumor morphology and its underlying genotype. This correlation has empowered recent studies from the Nikiforov group and others that demonstrated that genotyping improves the accuracy of thyroid FNA cytology diagnoses (3). Thus, genotyping of these genes is a powerful tool that will permit improved diagnosis and even imparts some prognostic information, as \textit{BRAF} mutation is a risk factor for PTC recurrence (reviewed in (4)). However, the cancer genome is extremely complex and genotyping for these genes does not provide a complete picture of the genetics of these tumors. This fact is nicely illustrated in the following figure from the review of Xing (4) that shows secondary events in \textit{BRAF}-mutant PTC that lead to tumor progression and invasiveness.

![Genome-wide Studies Diagram](image)

**Genome-Wide Studies**

Over the last decade, we have witnessed great advances in genomic science. The availability of genome-wide data is radically changing almost every field of biology, from cancer genetics to agriculture to human evolution. In addition, breakthroughs in high-throughput molecular biology techniques, such as DNA microarrays, and next-generation DNA sequencing, have leveraged genomic information to yield very interesting and informative studies. For instance, the genome of a single small cell neuroendocrine lung carcinoma was published recently, revealing literally thousands of DNA alterations and a mutational profile of tobacco smoking (5).

Genome-wide studies of follicular cell thyroid tumors have been more modest, but nonetheless informative. A full representation of the field is beyond the scope of this handout. This field was reviewed in 2008 (6). Selected studies will be presented.

Work from my research group at the University of Michigan in collaboration with Yuri Nikiforov has focused on genome-wide gene expression studies of follicular cell thyroid tumors using DNA microarrays.
We examined 97 follicular cell tumors and were able to recapitulate the overall established classification of thyroid tumors, thereby confirming the biologic significance of the gene expression measurements. Using the dataset, we showed that the tumors were broadly divided into 2 groups, those with follicular architecture and those with papillary architecture. Thus, with this approach, the follicular variant of PTC tumors (FV-PTC) were more closely related to the other follicular-patterned tumors (follicular adenoma (FTA) and follicular carcinoma (FTC)) than classical and tall cell type PTC. Thus, based solely on global gene expression, one would be tempted to classify the FV-PTCs within the umbrella of FTC rather than PTC.

Using this same dataset, we also showed that, within the group of PTC, global gene expression was strongly correlated to both tumor morphology and tumor genotype, as shown in the following principal components analysis (PCA) from (7):

![PCA plots](image)

a, PCA with tumor morphology  

b, PCA with RET/PTC, RAS and BRAF genotype

Also using this dataset, we showed that global gene expression in FTCs with the PAX8/PPARγ translocation was distinctly different from other types of follicular cell tumors, demonstrating that this translocation drives expression of many PPARγ target genes (8).

Collectively, these gene expression studies show a striking degree of correlation between a follicular cell tumor’s morphology, its genotype and its gene expression profile and offer a unifying scheme for the classification of these tumors.

Several studies have focused on gene expression profiling as a diagnostic aide using mRNA profiling of follicular tumors (9, 10). A more complete discussion of these studies can be found at (6).

**MicroRNA Studies**

At least 8 studies have examined expression of panels of miRNAs in thyroid tumors with the ultimate goal of defining specific miRNAs that possess diagnostic power (literature review can be found in (11). While several miRNAs with differential expression have been identified, no study has yet defined a panel that is ready for diagnostic use. Consistently identified differentially expressed miRNAs include 146b, 221, and 222. Over the next few years, it will become clearer what role miRNA profiling can play in the diagnosis of follicular cell thyroid tumors.

**What’s Still Needed**
Genomic evaluation of follicular cell tumors is still in its infancy. With the advances of next generation sequencing, it is now possible to sequence the genome of individual cancers (5, 12-14). Such studies have been applied to several tumor types and have greatly expanded the spectrum of mutations observed. It is fully anticipated that application of this approach to follicular cell tumors will lead to advances in the classification and diagnosis of these diagnostically challenging tumors.

Appendix

The paper by Stratton, Campbell and Futreal (Nature 458: 79-724) provides the following definitions of driver and passenger mutations:

“All cancers arise as a result of somatically acquired changes in the DNA of cancer cells. That does not mean, however, that all the somatic abnormalities present in a cancer genome have been involved in development of the cancer. Indeed, it is likely that some have made no contribution at all. To embody this concept, the terms ‘driver’ and ‘passenger’ mutation have been coined.

“A driver mutation is causally implicated in oncogenesis. It has conferred growth advantage on the cancer cell and has been positively selected in the microenvironment of the tissue in which the cancer arises. A driver mutation need not be required for maintenance of the final cancer (although it often is) but it must have been selected at some point along the lineage of cancer development.”

“A passenger mutation has not been selected, has not conferred clonal growth advantage and has therefore not contributed to cancer development. Passenger mutations are found within cancer genomes because somatic mutations without functional consequences often occur during cell division. Thus, a cell that acquires a driver mutation will already have biologically inert somatic mutations within its genome. These will be carried along in the clonal expansion that follows and therefore will be present in all cells of the final cancer.”

From Stratton, Campbell and Futreal (Nature 458: 79-724).

References

1. Rosai J 2010 The Encapsulated Follicular Variant of Papillary Thyroid Carcinoma: Back to the Drawing Board. Endocr Pathol


