Application of Immunohistochemistry to Thyroid Neoplasms

Sandra Fischer, MD; Sylvia L. Asa, MD, PhD

Objectives.

- Thyroid lesions with nodular architecture and follicular pattern of growth often pose difficulties in accurate diagnosis during the assessment of cytologic and histologic specimens. The diagnosis of follicular neoplasm on cytology or of follicular tumor of uncertain malignant potential on histology is likely to cause confusion among clinicians and delay effective management of these lesions. Occasionally, thyroid tumors represent unusual or metastatic lesions and their accurate diagnosis requires immunohistochemical confirmation.

Objective.—To review the literature on the applications of immunohistochemistry in the differential diagnosis of thyroid tumors.


Conclusions.—Our review supports the use of ancillary techniques involving a panel of antibodies suitable for immunohistochemistry and molecular analysis in the assessment of thyroid nodules. These tools can improve diagnostic accuracy when combined with standard morphologic criteria.

(Arch Pathol Lab Med. 2008;132:359–372)
molecular events involved in thyroid tumor initiation and progression, resulting in genomic instability and the capacity for independent cellular growth, invasion, and metastasis.\(^9\) It seems unrealistic to expect a single tool, in the form of a magic biomarker, to be able to effectively resolve the diagnostic dilemmas in thyroid pathology. Each marker differentially expressed in tumorous and nontumorous tissues represents a snapshot of the molecular events succeeding in the tissue environment. The amount of information a single marker offers is often insufficient to understand tumor biology or to render accurate diagnosis. The use of combined immunohistochemical markers as a panel seems to be an alternative to aid some of the diagnostic challenges in surgical pathology and cytopathology of thyroid specimens.\(^7,10,11\) Most important, genomic and proteomic technologic approaches are being developed to introduce molecular signatures capable of separating benign from malignant thyroid tumors and, in the last group, to distinguish tumors with indolent and aggressive behavior.\(^12-14\)

**WHEN SHOULD ANCILLARY TECHNIQUES BE APPLIED?**

Nodular tumors exhibiting a predominantly follicular architecture are the most common type of lesion of the thyroid. In most cases, a diagnosis can be readily assessed without difficulty based on histologic and clinical evidence. For instance, hyperplastic nodules are usually associated with nodular goiter and can be promptly recognized by their variability of follicular size and degenerative changes including fibrosis, hemorrhage, and cyst formation.\(^13,14\) Follicular adenomas, usually presenting as single nodules, are separated from the normal thyroid parenchyma by an intact fibrous capsule; they usually exhibit a predominance of microfollicles or macrofollicles and lack vascular invasion.\(^4\) Hyperplastic nodules (also called adenomatoid nodules) with an exclusive microfollicular or macrofollicular architecture and adenomas with incomplete or disrupted capsules can pose difficulties in diagnosis.\(^15\) In addition, atypical follicular adenomas comprise a group of noninvasive lesions with increased cellularity, nuclear atypia, and/or mitotic activity, in which tumor necrosis and infarction can often be demonstrated.\(^17\) Another controversial entity, the so-called hyalinizing trabecular adenoma (HTA), shares morphologic features with PTC.

Examples of malignant follicular patterned lesions of the thyroid that may impose assessment difficulties to the diagnosis include encapsulated or minimally invasive follicular carcinoma, clear cell carcinomas that can mimic metastatic carcinomas, follicular and oncocytic variants of papillary carcinoma, follicular variant of medullary carcinoma, and the rare mixed follicular-medullary tumors.\(^7,11\) Traditionally, the diagnosis of follicular carcinoma relies on demonstrating capsular and/or vascular invasion.\(^4\) The interpretation of what constitutes capsular and vascular invasion may vary among pathologists.\(^15,18,19\) The term well-differentiated tumor of undetermined malignant potential has been used by some authors to designate follicular carcinomas presenting only minimal capsular invasion.\(^20\) The basis of the nomenclature relies on the fact that this tumor category shows indolent behavior. In contrast, follicular carcinomas presenting vascular invasion with or without capsular invasion tend to recur and spread to distant organs more often and have been called grossly encapsulated angiinvasive follicular carcinoma.\(^15,17\) Clear cell tumors can be diagnosed as follicular adenomas or follicular carcinomas, but even minor atypical features should prompt the consideration of metastasis from renal, lung, or adrenal carcinoma.\(^21\) Follicular variant of papillary thyroid carcinoma (FVPTC) can be difficult to diagnose when tumor cells lack all of the obvious nuclear features of papillary carcinoma.\(^15,22\) Even more problematic is the interpretation of unifocal or multifocal nuclear changes of papillary carcinoma in nodules with a predominantly bland cytology.\(^15,23\) Observer variation in the diagnosis of FVPTC has been demonstrated even by experienced pathologists.\(^24\) The most important histologic features used to identify FVPTC include the presence of cytoplasmic invaginations, abundant nuclear grooves, and ground glass nuclei.\(^24\) Some authors recommend the use of strict criteria when evaluating a potential FVPTC and propose a combination of major and minor histologic features. Among the latter are the presence of abortive papillae, elongated or irregularly shaped follicles, dark-staining colloid, rare nuclear pseudoinclusions, and multinucleated histiocytes in the follicular lumens.\(^25\) The diagnosis of oncocytic follicular variant of papillary carcinoma remains controversial. Most of these lesions present irregular follicles with hyperendocrine colloid and the nuclear features of papillary carcinoma, which can be often obscured by hyperchromasia, and prominent nucleoli that characterize oncocyctic change.\(^26\) True follicular differentiation may occur in medullary carcinomas of the thyroid gland and mixed follicular-medullary tumors.\(^4\) Nuclear features suggestive of neuroendocrine differentiation and cytoplasmic granularity are the most relevant morphologic findings, but complementary immunohistochemical analysis is required to confirm the diagnosis.\(^15\)

Fine-needle aspiration cytology (FNAC) has greatly improved the clinical management of thyroid nodules. However, FNA has inherent limitations related not only to inadequate sampling but also, most importantly, to its inability to distinguish between benign and malignant follicular lesions in the absence of nuclear features of papillary carcinoma. The indeterminate diagnosis of follicular neoplasm encompasses a number of heterogeneous thyroid lesions including cellular adenomatoid nodule, follicular adenoma, and follicular carcinoma.\(^11,22\) Additionally, the interpretation of FVPTC cytology can be difficult when prominent classic nuclear features of PTC are absent. In such cases, a preoperative diagnosis of “follicular lesion suggestive of PTC” results in conservative surgical assessment until a definitive diagnosis can determine the appropriate treatment.

Several immunohistochemical markers using different antibodies, alone or combined in panels, have been postulated to improve diagnostic accuracy of follicular-patterned thyroid lesions.\(^5,10\) They belong to different categories and are involved in cell adhesion (galectin-3, E-cadherin, fibronectin [FN]), receptor signaling (RET), gene transcription control (thyroid transcription factor 1 [TTF-1]), secretion (thyroglobulin [TG], calcitonin, carcinoembryonic antigen [CEA]), cell cycle regulation (p27, cyclin D1), and cellular structure (cytokeratin [CK] 19). They are detected in different cellular compartments such as membrane and/or cytoplasm (Hector Battifora mesothelial cell) 1 [HBME-1], β-catenin and nucleus (p53).
RET

The RET proto-oncogene (c-RET) encodes a tyrosine-kinase receptor protein whose ligands belong to the family of glial cell line–derived neurotropic factors. In conjunction with membrane-bound, ligand-binding glial cell line-derived neurotropic factor receptors, RET operates as an intracellular signal-transducing element. RET/PTC is a rearranged version of RET. Somatic rearrangements of RET were identified in PTC before RET was recognized as the susceptibility gene for multiple endocrine neoplasia type 2. In multiple endocrine neoplasia type 2, point mutations result in constitutive activation of RET, resulting in medullary thyroid carcinomas; there is no role for immunohistochemistry in this setting. In contrast, RET rearrangements are considered specific for PTC, and they occur when RET undergoes a translocation fusing its tyrosine kinase domain to a variety of other 5′ elements, thereby removing the promoter, the extracellular ligand-binding domain, and the membrane-anchoring domain of RET. RET/PTC-1, -2, and -3, and a number of other less common rearrangements resulting in RET oncogene activation, play a key role in the pathogenesis of PTC. The constitutive activation of the tyrosine domain in the carboxyl-terminal end of RET/PTC induces signaling pathways within thyrocytes and causes cellular transformation in transgenic mice. RET/PTC is also considered to lead to the characteristic nuclear features of PTC through alteration of the nuclear envelope and chromatin structure. There is a wide variation in the reported frequency of RET activation, which is related to the sensitivity of the detection techniques and exposure to ionizing radiation. RET/PTC-1 and RET/PTC-3 are the most common types of RET rearrangements reported in non-radiation-induced PTC (40% and 15% of cases, respectively). The identification of RET/PTC rearrangements has increased the ability to diagnose PTC. The utility of aberrant RET expression by rearrangement as a diagnostic marker in borderline thyroid lesions and preoperative evaluation of thyroid aspirates is supported by its high specificity for PTC. RET immunostaining has been shown to be useful in the assessment of thyroid lesions with incomplete and/or focal features of PTC in which positive staining has been demonstrated in more than 50% of cases in close parallel with the morphologic features suggestive of PTC.

In the same study, no RET/PTC-1 or RET/PTC-3 rearrangements were detected by reverse transcription-polymerase chain reaction (RT-PCR) in the tumor areas lacking the cytologic alterations. RET protein detection by immunohistochemistry has also been reported in the so-called HTAs, and this finding was confirmed by detection of RET/PTC-1 rearrangements by RT-PCR, suggesting that HTA may represent a variant of PTC. Similarly, it has been shown that oncotypic tumors that exhibit nuclear features of PTC also harbor RET/PTC rearrangements. RET protein–positive immunostaining can also be demonstrated in the oncotypic cells of this subset of tumors, supporting the revised classification system for oncotypic tumors that recognizes an oncotypic follicular variant of PTC.

Although RET expression is a valuable diagnostic tool for PTC, it has no value as a predictive marker. A relatively low prevalence of positive RET staining has been reported in poorly differentiated and anaplastic thyroid carcinomas. This is consistent with the biology of the rearrangements that rely on promoters of genes expressed by differentiated thyroid follicular cells; dedifferentiation results in down-regulation of expression and loss of RET/PTC gene expression by RT-PCR as well as protein expression by immunohistochemistry.

Although close correlation between RT-PCR detection of RET/PTC and immunohistochemical detection of RET protein product has been reported, these results were highly dependent on the availability of appropriate antisera; however, availability has been inconsistent, and therefore molecular testing appears to be superior to immunohistochemistry to identify these rearrangements. Currently a number of monoclonal antibodies directed against the RET carboxy-terminus are commercially available, but most fail to reproduce the diffuse cytoplasmic positivity observed with previous polyclonal antisera that are no longer commercially available.

CYTOKERATIN 19

Different subtypes of keratin filaments are grouped according to molecular weight. High-molecular-weight CKs (CK1, CK4, CK10, and CK13) are detected in stratified squamous epithelium. Simple or glandular epithelium expresses CK7, CK8, CK18, and CK19. The thyroid gland has been extensively studied with various antibodies to CKs in an attempt to identify differential expression patterns in normal parenchyma, benign nodules, and malignant tumors. Papillary carcinomas have been shown to express strong and diffuse immunoreactivity for CK7, CK18, and CK19 in 80% to 100% of cases. High-molecular-weight CK using the antibody 34B12 has also been demonstrated in cases of PTC. Similar but less intense staining patterns are seen in follicular variant PTC using CK17 and CK20 antibodies. The expression of CK7, CK18, and CK19 has been shown to be less frequent in cases of poorly differentiated carcinomas (60%, 60%, and 40%, respectively). Squamoid and giant cell–solid epithelioid variants of anaplastic thyroid carcinoma frequently express CK7, CK8, and CK18. In contrast, these keratins and CK19 are rarely detected in the spindle cell sarcomatoid variant of anaplastic thyroid carcinoma.

Because CK19 detection in follicular adenomas and follicular carcinomas is often less intense and more focal than in PTC, this keratin has become one of the most commonly used to investigate thyroid lesions. Several authors emphasize the importance of the distribution and intensity of CK19 staining as the most critical aspects of accurate interpretation. Normal thyroid follicular epithelium is often negative, although focal staining for CK19 is usually identified in the compressed thyroid parenchyma surrounding nodules and in follicular cells within lymphocytic thyroiditis. This pattern of staining is consistent with the intense pattern of staining seen in reactive follicular epithelium within thyroid nodules around the site of degeneration, especially at the site of a previous needle biopsy. However, the finely dispersed positivity seen in the cells of PTC is distinctive (Figure 1). Although this feature is usually diffuse throughout the lesion, focal staining for CK19 does not rule out a diag-
Immunostaining for cytokeratin 19 in a follicular lesion of thyroid demonstrates a diffuse cytoplasmic reaction in tumor cells. The intensity increases in invasive cells compared with those lining follicles. The staining is not dark and intense as is focally seen in reactive epithelium. This feature is characteristic of a subset of papillary thyroid carcinomas (original magnification ×300).

Figure 2. Galectin-3 is overexpressed in thyroid malignancies, where it is localized to both cytoplasm and nuclei (original magnification ×400).

Figure 3. A, Papillary and follicular carcinomas exhibit strong staining for Hector Battifora mesothelial (cell) 1 (HBME-1) that can be intensely positive with diffuse cytoplasmic reaction and focal apical intensification of the reaction (original magnification ×400). B, In some lesions the staining for HBME-1 can be more subtle with predominant apical reactivity that can be misinterpreted as nonspecific reaction of colloid (original magnification ×200).

nosis of PTC, particularly in nodules with nuclear features of PTC that are seen focally. CK7 and CK18 have been detected in a high percentage of so-called HTAs. Variable expression of CK19 (50%–100%) in HTA has been interpreted by some investigators as proof that these tumors are benign, whereas others use this as evidence that they should be classified as a variant of papillary carcinoma. Medullary carcinomas have been reported to express strong positive staining for CK7 and CK18 in 77% of cases and only focal staining for CK19 in 69% of lesions.

CK19 has also been considered by many investigators to be a useful ancillary tool for the diagnosis of papillary carcinoma in FNAC, especially in cytologically suggestive but indeterminate cases. The reported sensitivity and specificity using CK19 as a single marker is as high as 92% and 97%, respectively. In addition, stronger reactivity has been reported in methanol-fixed thin-layer preparations. The use of CK19 immunolocalization in cell block preparation of thyroid aspirates has also been reported to aid in accurate diagnosis of malignancy in cytologically equivocal cases of PTC. A panel of markers including CK19 and galectin-3 was reported as reaching 100% of both specificity and sensitivity in the management of thyroid lesions with a cytologic diagnosis of follicular oncocyty tumors. Given the well-known positivity of CK19 in reactive atypia and chronic lymphocytic thyroiditis that can mimic PTC, the use of CK19 on cytologic specimens should be interpreted with great caution, and many diagnosticians, the authors included, do not endorse this application.

GALECTIN-3

Galectin-3 (31-kd molecular weight) is one of the members of a family of non-integrin β-galactoside–binding lect-
Table 1. Immunohistochemical Detection of Galectin-3 in Thyroid Surgical Specimens*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0 (0)</td>
<td></td>
<td></td>
<td>0 (0)</td>
<td></td>
<td></td>
<td>0/75 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2/4 (50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0/7/100</td>
<td>16/29 (55)</td>
<td>15/18 (50)</td>
<td>195/201 (97)</td>
<td>39/39 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>0 (0)</td>
<td>3/8 (37)</td>
<td>4/19 (21)</td>
<td>5/132 (4)</td>
<td>3/50 (6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTC</td>
<td>18/18 (100)</td>
<td>22/43 (64)</td>
<td>19/20 (95)</td>
<td>22/24 (92)</td>
<td>63/67 (94)</td>
<td>169/202 (84)</td>
<td>195/201 (97)</td>
<td>39/39 (100)</td>
<td></td>
</tr>
<tr>
<td>FTC</td>
<td>4/8 (50)</td>
<td>2/3 (67)</td>
<td>7/10 (70)</td>
<td>4/9 (44)</td>
<td>4/6 (67)</td>
<td>7/11 (64)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVPFTC</td>
<td></td>
<td>5/8 (62)</td>
<td></td>
<td>4/1 (100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDTC</td>
<td>2/3 (67)</td>
<td></td>
<td></td>
<td>13/20 (65)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATC</td>
<td>5/5 (100)</td>
<td></td>
<td></td>
<td>18/20 (90)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values are number/total number (percent). Ellipses indicate not addressed in this article; CLT, chronic lymphocytic thyroiditis; NH, nodular hyperplasia; FA, follicular adenoma; PTC, papillary thyroid carcinoma; FTC, follicular thyroid carcinoma; OVPFTC, oncocytic variant of papillary and follicular thyroid carcinoma; PDTC, poorly differentiated thyroid carcinoma; and ATC, anaplastic thyroid carcinoma.

**Table 2.**

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>CLT</th>
<th>NH</th>
<th>FA</th>
<th>PTC</th>
<th>FTC</th>
<th>OVPFTC</th>
<th>PDTC</th>
<th>ATC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45%</td>
<td>21%</td>
<td>9%</td>
<td>65%</td>
<td>90%</td>
<td>60%</td>
<td>70%</td>
<td>67%</td>
<td>55%</td>
</tr>
</tbody>
</table>

The table presents the frequencies of galectin-3 detection in different thyroid lesions, ranging from 45% to 90%.

**HBM-E-1**

HBME-1 is a monoclonal antibody that recognizes an unknown antigen in the microvilli of mesothelioma cells, normal tracheal epithelium, and adenosarcoma of the lung, pancreas, and breast.7,27,45,75 HBME-1 has also been reported by several investigators to be a useful marker of malignancy and can be a helpful tool for classical PTC, but it cannot be considered a diagnostic marker alone. Caution has to be taken when interpreting positive results in the absence of unequivocal morphologic features of PTC because of the possibility of false positives. False-negative results in unconventional forms of PTC should also be considered when evaluating unconventional forms of PTC.

**Conclusion**

226 specimens of thyroid nodules obtained preoperatively by ultrasound-guided FNA (188 benign lesions and 34 carcinomas), the sensitivity, specificity, positive predictive value, and diagnostic accuracy of galectin-3 immunodetection were 100%, 98%, 92%, and 99%, respectively.11 In addition, another study of 125 thyroid aspirates (50 follicular adenomas, 33 follicular carcinomas, and 42 papillary carcinomas) showed that galectin-3 has a sensitivity, specificity, positive predictive value, and diagnostic accuracy of 92%, 94%, 95.8%, and 92.8%, respectively.33 In contrast, a lower specificity (52%) was reported in a smaller series in which 11 of 44 follicular adenomas (31/35 follicular carcinomas) exhibited positive staining for galectin-3.73

It is evident from these data that galectin-3 may be a useful tool for classical PTC, but it cannot be considered a diagnostic marker alone. Caution has to be taken when interpreting positive results in the absence of unequivocal morphologic features of PTC because of the possibility of false positives. False-negative results in unconventional forms of PTC should also be considered when evaluating unconventional forms of PTC.
Figure 4. Nuclear reactivity for peroxisome proliferator-activated receptor γ is almost undetectable in most thyroid follicular epithelial cells. In this invasive malignancy that harbors a t(2;3)(q13;p25) rearrangement, there is marked up-regulation of the protein product that can be identified by immunostaining (original magnification ×300).

Figure 5. Well-differentiated thyroid cells exhibit a discrete membranous staining pattern of β-catenin (A), whereas poorly differentiated malignancies that demonstrate dyscohesive growth lose the membrane staining pattern and instead demonstrate nuclear translocation of that protein (B) (original magnification ×400).

Figure 6. A papillary carcinoma that exhibits abundant nuclear reactivity for cyclin D1 is likely to be an aggressive tumor with great potential for metastatic spread (original magnification ×400).
Table 2. Immunohistochemical Detection of Hector Battifora Mesothelial (Cell) 1 (HBME-1) in Thyroid Surgical Specimens*

<table>
<thead>
<tr>
<th>Normal</th>
<th>NH</th>
<th>FA</th>
<th>PTC</th>
<th>FTC</th>
<th>OVPFTC</th>
<th>PDTC</th>
<th>ATC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/59 (0)</td>
<td>0/35 (0)</td>
<td>0/35 (0)</td>
<td>76/138 (55)</td>
<td>2/4 (50)</td>
<td>2/7 (29)</td>
<td>4/6 (67)</td>
<td>1/2 (50)</td>
</tr>
<tr>
<td>0/62 (12)</td>
<td>17/62 (27)</td>
<td>35/36 (97.2)</td>
<td>33/39 (84.6)</td>
<td>50/50 (100)</td>
<td>7/10 (20)</td>
<td>0/2 (0)</td>
<td></td>
</tr>
<tr>
<td>1/29 (3)</td>
<td>2/21 (10)</td>
<td>57/67 (85)</td>
<td>3/6 (50)</td>
<td>1/8 (13)</td>
<td>1/9 (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/15 (6.6)</td>
<td>2/50 (4)</td>
<td>65/67 (97)</td>
<td>30/30 (100)</td>
<td>4/6 (66.6)</td>
<td>11/12 (91.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>. . . .</td>
<td>. . . .</td>
<td>14/14 (100)</td>
<td>. . . .</td>
<td>. . . .</td>
<td>6/17 (39)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>. . . .</td>
<td>. . . .</td>
<td>37/39 (94.8)</td>
<td>. . . .</td>
<td>. . . .</td>
<td>. . . .</td>
<td></td>
<td></td>
</tr>
<tr>
<td>. . . .</td>
<td>. . . .</td>
<td>11/33 (34)</td>
<td>. . . .</td>
<td>. . . .</td>
<td>. . . .</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values are number/total number (percent). Ellipses indicate not addressed in this article; NH, nodular hyperplasia; FA, follicular adenoma; PTC, papillary thyroid carcinoma; FTC, follicular thyroid carcinoma; OVPFTC, oncocytic variant of papillary and follicular thyroid carcinoma; PDTC, poorly differentiated thyroid carcinoma; and ATC, anaplastic thyroid carcinoma.

Studies have varied between 50% and 100% (mean, 75%). Small series of oncocytic variant of papillary and follicular carcinoma have demonstrated positive HBME-1 staining in 13% to 66% of cases. Poorly differentiated and anaplastic carcinomas also often express HBME-1 (67%–91% and 0%–50%, respectively). A few studies have shown HBME-1 staining in follicular tumors of uncertain malignant potential that had either questionable vascular/capsular invasion or incomplete nodule formation. COX-2 expression is induced by cytokines, hormones, inflammatory mediators, and mitogens. Several reports have shown COX-2 up-regulation in human cancers, including tumors of the lung, prostate, breast, colon, and pancreas. COX-2 promotes tumorigenesis by stimulation of cell growth, by induction of angiogenesis, and as an inhibitor of apoptosis. Recently, COX-2 has been detected in the thyroid, although the expression varies widely in tumors. Papillary and follicular carcinomas of the thyroid show a significantly higher perinuclear cytoplasmic immunoreactivity, compared with normal follicular cells and follicular adenomas. The sensitivity for PTC and FTC in different series has ranged between 70% to 90% and 26% to 93%, respectively. In one study, a significantly higher percentage of papillary microcarcinomas expressed COX-2 in comparison with all FTCs. In another study, COX-2 expression was reduced in tumors associated with older age, larger size, advanced stage, satellite tumors, and scirrhous or trabecular patterns of growth. These findings may indicate up-regulation of COX-2 in early phases of tumor progression. COX-2 has also been reported in poorly differentiated and anaplastic carcinomas and medullary carcinomas but was not detected in nonneoplastic C cells. Follicular adenomas variably express COX-2 (0%–28%), and it seems to be absent in hyperplastic nodules. Follicular cells in cases of Hashimoto thyroiditis stain positively for COX-2, and weak immunoreactivity has been reported in up to 17% of normal thyroid tissue samples. Thus, COX-2 appears to be a novel biologic marker that can shed insight into tumorigenic mechanisms, but it does not seem to be valuable as a diagnostic marker. Its potential for prognostication remains to be proven because loss in a differentiated tumor may have significance.

**PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ**

PAX8-PPARγ1 rearrangements were initially reported in a subset of angioinvasive follicular carcinomas as a result of the translocation t(2;3)(q13;p25), which leads to fusion of the DNA-binding domains of the thyroid transcription factor PAX8 to domains A to F of the PPARγ1. The same study, PAX8-PPARγ1 messenger RNA (mRNA) and protein were absent in hyperplastic nodules, follicular adenomas, and papillary carcinomas, suggesting that PAX8-PPARγ1 might be useful in the assessment of FTC. Since then, several studies have confirmed the presence of PAX8-PPARγ1 expression by RT-PCR and strong nuclear staining by immunohistochemistry (Figure 4) in 53% to 69% of follicular carcinomas, as well as in a small number of papillary carcinomas, and its absence in nodular hyperplasia. Some studies have confirmed an association with vascular invasion. However, PAX8-PPARγ1...
mRNA and protein have been demonstrated in 8% to 27% of follicular adenomas, suggesting that this marker might not be suitable to separate follicular adenoma from follicular carcinoma.91–94 In addition, moderate to strong immunoreactivity for PPARγ has been detected in nonlesional surrounding tissue associated with chronic lymphocytic thyroiditis.95 Thus, the value of this marker remains unproven. However, the data accumulating suggest that, in known malignancies, the presence of PPARγ up-regulation may predict a worse prognosis.

E-CADHERIN

Caderhins are cell-cell adhesion molecules involved in the morphogenesis of developing tissues and maintenance of adult solid tissues. Cadherins have an intracellular domain that binds to catenins. Loss of cell-cell adhesion is a major cancer hallmark that correlates with loss of differentiation and aggressive tumor behavior. Normal thyroid follicular cells express uniformly high levels of E-cadherin mRNA and have a strong cell surface pattern of staining.96 E-cadherin staining is variably reduced in well-differentiated thyroid carcinomas and frequently absent in poorly differentiated and anaplastic carcinomas.97–99 Loss of E-cadherin expression is an adverse prognostic factor in differentiated thyroid carcinomas.96,97

β-CATENIN

β-Catenin is a 94-kd protein that forms cytoplasmic/membranous-bound complexes with E-cadherin and is involved in the assembly of the zonula adherens.99 When activated, it translocates to the nucleus where it promotes tumor growth through activation of the wnt-signaling pathway.99 Strong membranous immunoreactivity with minimal β-catenin cytoplasmic staining is observed in normal follicular cells.85,100 Thyroid tumors express different patterns of immunoreactivity, including strong to weak membranous positivity reported in follicular adenomas and well-differentiated thyroid carcinomas (Figure 5, A) and loss of membranous staining with nuclear and cytoplasmic staining in poorly differentiated and anaplastic carcinomas (Figure 5, B). Along with E-cadherin, loss of membrane β-catenin immunostaining is an indicator of loss of differentiation and adverse prognosis. Aberrant nuclear immunoreactivity of β-catenin is associated with stabilizing CTNNB1 exon 3 mutations that are found almost exclusively in poorly differentiated and anaplastic carcinomas.100 The cribriform-morular variant of PTC, which is pathognomonic of familial adenomatous polyposis–associated thyroid carcinoma, has been reported to demonstrate cytoplasmic and nuclear accumulation of β-catenin and CTNNB1 exon 3 mutations.100,102

FIBRONECTIN-1

Fibronectins are multifunctional adhesive glycoproteins found in the extracellular matrix and body fluids. They have affinity for collagen, fibrin, heparin, and cell surfaces and are involved in various biologic processes including cell adhesion, migration, and tumor progression. Plasma FN is produced by adult hepatocytes. Oncofetal FNs are cellular isoforms containing the extracellular domain A or B and III connecting segments. Oncofetal FNs are highly expressed in fetal and neoplastic tissues, including thyroid follicular cell–derived tumors.67,103–106 Oncofetal FN has been proposed as a potential useful adjunct for preoperative diagnosis of thyroid nodules, based on findings of initial reports demonstrating a restricted expression of oncofetal FN mRNA in papillary and anaplastic carcinomas, compared with normal thyroid tissues, hyperplastic lesions, follicular adenomas, and carcinomas, using an RT-PCR based approach.106–109 However, other groups have identified oncofetal FN in chronic lymphocytic thyroiditis, follicular adenomas, and follicular and medullary carcinomas.67,105

Fibroblast emerged as a potential marker of thyroid carcinoma in microarray studies in which it was reported to be up-regulated compared with normal tissue.110–112 An immunohistochemical panel consisting of FN, galectin-3, and HBME-1 has been reported to be effective in the diagnosis of follicular cell–derived thyroid tumors.67 However, FN expression is reduced in more aggressive cancers and in the invasive components of differentiated carcinomas.104,113 In fact, modulation to up-regulate FN may represent a therapeutic target to prevent invasion and metastasis in thyroid carcinoma.114

Fibroblast immunoreactivity is also observed in extra-cellular fibrosis in goiters; cytoplasmic and membranous staining is seen in reactive follicular cells associated with hemorrhage and fibrin deposition.99 These findings may explain false-positive fine-needle aspirates examined by molecular-based studies because of contamination with fibroblasts and reactive follicular cells.115

CD44v

CD44, also known as extracellular matrix receptor III, hyaluronate receptor, and heparan sulfate proteoglycan, is a family of immunologically related integral membrane glycoproteins that bind hyaluronic acid. CD44 mediates cell-cell and cell-matrix interactions through its affinity for hyaluronic acid and possibly also through its affinity for other ligands, such as osteopontin, collagens, and matrix metalloproteinases. Adhesion with hyaluronic acid plays an important role in cell migration, tumor growth, and progression. CD44 family members are highly polymorphic because of numerous alternative splicing and post-translational modification events. Isoforms sharing CD44 variant exon 6 are known to confer tumor invasiveness and metastatic potential in malignant rat cell lines.116–118 CD44v6 has been comparatively assessed on thyroid lesions by immunohistochemistry in surgical specimens and fine-needle aspirates.11,73,119 Intense membrane staining has been demonstrated in benign lesions, including hyperplastic nodules (40%) and follicular adenomas (30%–43%).11,119 Well-differentiated papillary and follicular carcinomas show the highest immunoreactivity for CD44v6 (75%–90% and 90%–100%, respectively).11,73,119 It has also been detected in poorly differentiated and anaplastic carcinomas, oncocytic variant follicular and papillary carcinomas, and medullary carcinoma.11 These findings suggest that CD44v6 may be associated with deregulated follicular proliferation, rather than malignant transformation, and should not be used as a single marker to discriminate benign from malignant thyroid lesions.

THYROID PEROXIDASE

Thyroid peroxidase (TPO) is a thyroid-specific enzyme involved in the synthesis of thyroid hormone. Gene suppression and mutations of the TPO gene were reported in some differentiated thyroid carcinomas.120,121 Reduced TPO immunoreactivity has been proposed by some investigators as a marker to distinguish benign from malignant
thyroid tumors at preoperative assessment of thyroid nodules by FNAC.120,122–125 In these studies, the absence of TPO immunoreactivity in more than 80% of cells could accurately demonstrate malignancy with sensitivities between 97% and 100% and specificities ranging from 68% to 90%. More recently, these numbers have been confirmed by other investigators as applicable to papillary but not to follicular carcinomas.65,126,127 The reason for this discrepancy is a high overlap of TPO immunoreactivity patterns between follicular adenomas and carcinomas. In addition, preserved immunoreactivity for TPO, as occurring in follicular carcinomas, does not rule out malignancy. The use of TPO in combination with other immunohistochemical markers has been reported as useful for the diagnosis of papillary carcinoma in surgical thyroid specimens.65

Cbp/p300-INTERACTING TRANSACTIVATOR 1

Cbp/p300-interacting transactivator 1 (CITED1), which is also known as melanocyte-specific protein 1, is a nuclear protein involved in coregulation of transcription factors. CITED1 has been detected in the nucleus and cytoplasm of melanocytes, epithelial breast cells, and testicular germ cells. CITED1 gene overexpression has been demonstrated in papillary carcinomas of the thyroid by complementary DNA microarray analysis and RT-PCR.110–112 In addition, immunohistochemical studies have shown CITED1 activity in 87% to 93% of papillary carcinomas (all subtypes), 0% to 50% of follicular carcinomas, 10% to 16% of follicular adenomas, and 24% of nodular goiters.67,111,112,128 These findings indicate that it lacks specificity as a diagnostic modality, but some authors maintain that when used in combination with other antibodies including HBME-1, galectin-3, CK19, and FN, CITED1 may be helpful in the diagnosis of papillary carcinoma.67,128

CYCLIN D1

The CCND1 gene located on chromosome 11q23 encodes a nuclear protein that forms complexes with cyclin-dependent kinases 4 and 6, resulting in phosphorylation and inactivation of the retinoblastoma protein, allowing cell cycle progression from G0 to S phase. Normal thyroid follicular cells do not show immunoreactivity for cyclin D1. Cyclin D1 is axtarget for /H9252-b-catenin, and cyclin D1 overexpression is associated with /b-catenin alterations in thyroid cancer progression.129,130

Increased nuclear expression of cyclin D1 mRNA and protein has been demonstrated in benign and malignant tumors of the thyroid (Figure 6).131–133 Cyclin D1 overexpression in thyroid papillary microcarcinomas was found to be significantly higher in tumors larger than 5 mm.130 Malignant tumors exhibit the highest immunoreactivity for cyclin D1, particularly papillary carcinomas, although follicular adenomas have been reported to demonstrate increased expression for cyclin D1.132,133 Again, these data must be interpreted with caution because of the known interobserver variability in the classification of adenomas and carcinomas.

A significant correlation between cyclin D1 overexpression and the presence of regional lymph node metastasis has been observed in patients with papillary carcinomas, suggesting that immunodetection of cyclin D1 by immunohistochemistry could be a useful prognostic tool to distinguish indolent from metastatic papillary carcinoma.131,134

p27

The tumor suppressor gene p27kip1 located on chromosome 12p13 encodes a nuclear cyclin-dependent kinase I that inhibits the formation of cyclin D1/cyclin-dependent kinase complexes during G1 and early G1 phases of the cell cycle, thus preventing entry to S phase through activation of retinoblastoma protein. Normal thyroid follicular epithelium shows strong nuclear immunoreactivity for p27.135,136 In contrast, thyroid tumors have been shown to exhibit decreased reactivity for p27, with significant differences of p27 expression between follicular adenomas and follicular-derived carcinomas.135 In addition, some investigators have reported a higher immunoreactivity for p27 in the follicular variant of papillary carcinoma, in comparison with classical PTC.136 In another study, p27 detection by immunohistochemistry was useful in distinguishing papillary hyperplasia of Graves disease from papillary carcinoma.137

Additionally, metastasizing papillary carcinomas show significant loss of immunoreactivity for p27 compared with those that are not associated with regional lymph node metastasis (Figure 7), providing another potential tool to distinguish indolent from metastatic PTCs.138,139

p53

Mutations of TP53 represent late genetic events in thyroid carcinogenesis. As a result, accumulation of p53 can be detected by immunohistochemistry most often in anaplastic (Figure 8) and poorly differentiated thyroid carcinomas.140 Rarely, it can be seen in well-differentiated papillary and follicular carcinomas as well as in medullary carcinomas.141 Positive immunoreactivity for p53 is an independent prognostic factor for overall survival of patients with thyroid cancer.142,143

THYROID TRANSCRIPTION FACTOR 1

TTF-1 was identified in 1989 as a nuclear tissue-specific protein with DNA-binding activity that interacted with the TG gene in the rat.144 TTF-1 regulates gene expression in the thyroid, lungs, and diencephalon during embryogenesis. Importantly, TTF-1 together with PAX8 control the expression of TG, TPO, thyrotropin receptor and the sodium/iodide symporter, calcitonin, and major histocompatibility complex class I genes in the thyroid.144–146 In the lung, TTF-1 regulates the expression of surfactant proteins A, B, and C and Clara cell secretory protein genes.147–150 Because retained TTF-1 expression is highly specific for thyroid and lung tumors, it has been widely used to discern the primary site of tumor origin in patients with metastatic disease of unknown origin.151–154 TTF-1 immunoreactivity is detected in pulmonary tumors, including neuroendocrine tumors, and rarely in small cell carcinomas from other sites.153–156 In the thyroid, nuclear reactivity for TTF-1 is present in follicular cell–derived benign and malignant lesions (Figure 9) and medullary carcinomas.157–159 Poorly differentiated carcinomas often show decreased and focal staining for TTF-1, and most anaplastic carcinomas lack TTF-1 reactivity. When used in combination with TG, TTF-1 is an effective marker for thyroid origin.158 Lack of TTF-1 immunoreactivity in a thyroid tumor should prompt investigation of other differential diagnoses, including parathyroid tumors, paragangliomas, and metastatic lesions.
Figure 9. A clear cell tumor in thyroid can be a primary tumor, an intrathyroidal parathyroid lesion, or a metastasis from lung, kidney, adrenal, or other sites. The identification of strong nuclear thyroid transcription factor 1 indicates either a primary thyroid neoplasm or spread from a lung primary malignancy. In contrast, loss of this immunoreaction should prompt investigation of an alternative primary site (original magnification ×1100).

Figure 10. The identification of cytoplasmic thyroglobulin is the proof of thyroid origin in a clear cell tumor (original magnification ×400).

THYROGLOBULIN

Thyroglobulin is the primary product synthesized in the thyroid, and the macromolecular precursor of the iodinated thyroid hormones thyroxine (T4) and triiodothyronine (T3). TG gene expression is coordinately regulated by TTF-1, TTF-2, and PAX8.160 Thyroglobulin is a reliable tumor marker that can be detected in the serum of patients with residual disease after ablation of the thyroid by surgery and radiiodine and relapse after disease-free interval. Compared with TTF-1, TG is also a highly sensitive histogenetic marker for follicular cell origin (Figure 10), although patchy staining pattern, particularly in the less well-differentiated tumors, may produce less reliable results in small biopsies.158,161,162 Thyroglobulin is not produced by medullary carcinomas10; however, interpretation must be cautious because TG is well known to diffuse through local tissues, resulting in artefactual staining that can hamper the diagnosis of medullary carcinoma. Interpretation of immunoreactivity in these lesions must recognize the geographic nature of diffusion. Similarly, TG positivity in perithyroidal lymph nodes is not a reliable marker of metastasis without confirmation of CK and/or TTF-1 staining.

CALCITONIN

Medullary thyroid carcinoma (MTC) and C cells stain positively for calcitonin, a secreted protein produced by parafollicular C cells, which causes a rapid but short-lived drop in the level of calcium and phosphate in blood by promoting the incorporation of those ions in the bones. Calcitonin (Figure 11, A), together with CEA, chromogranin, synaptophysin, and calcitonin gene-related peptide are the most useful immunohistochemical markers for the diagnosis of MTC, especially when facing histologic subtypes such as the follicular, papillary, or encapsulated variants that can pose diagnostic difficulties with follicular cell–derived carcinomas and paraganglioma.21,163–166 Calcitonin is also a diagnostic marker to confirm C-cell hyperplasia (Figure 11, B), which is usually associated with familial medullary carcinoma.4,121,167

Although calcitonin immunoreactivity is highly specific for MTC, the staining pattern may be variable; although usually diffuse, it may be focal, with 25% or less of cells exhibiting cytoplasmic reactivity.168 Absence of calcitonin reactivity may occur in up to 5% of MTC cases.10 Alternatively, calcitonin and calcitonin gene-related peptide mRNAs can be demonstrated by in situ hybridization in cases in which conventional immunohistochemistry is not able to detect these markers.169,170

CARCINOEMBRYONIC ANTIGEN

Staining for CEA in thyroid has had historical difficulties because of nonspecific reaction with CEA-like substances. The advent of monoclonal antibodies has obviated the problems, and the use of monoclonal CEA for thyroid diagnosis is highly recommended in specific circumstances. Using these specific antibodies, thyroid follicular cells and tumor derived from them are negative. Thus, the finding of CEA immunoreactivity in a thyroid tumor should prompt the diagnosis of another entity, usually MTC but also other lesions such as thymic-derived lesions or metastatic carcinoma.

Diffuse cytoplasmic staining for CEA can be demonstrated in hyperplastic and neoplastic C cells by immunohistochemistry (Figure 12).171–173 Carcinoembryonic antigen is a reliable marker for the diagnosis of MTC, with a higher sensitivity than calcitonin. Reports of CEA immunoreactivity in a small number of papillary and follicular carcinomas are almost certainly because of technical or diagnostic error.21,174 Calcitonin is lost with dedifferentiation of MTC, whereas CEA expression is retained by these lesions; therefore, CEA is particularly helpful in the assessment of medullary carcinomas that lack or present only focal reactivity for calcitonin and should be used in combination with other markers including TG, chromogranin, and synaptophysin.10,21

In addition, a wide number of immunohistochemical markers that indicate neuroendocrine differentiation have been shown to be present in medullary carcinoma, including serotonin, somatostatin, adrenocorticotropic hormone, gastrin-releasing peptide, and others.175–180
CONCLUSIONS

Most of the neoplastic lesions originated from the thyroid gland are diagnosed based on well-characterized histologic features. However, there is a subset of tumors with follicular architecture that lack unequivocal features of malignancy, thus posing difficulties in the distinction of benign and malignant conditions. Some tumors in the thyroid are not derived from follicular thyroid epithelium. In such cases, the use of ancillary techniques including immunohistochemistry and molecular analysis can significantly improve diagnosis. However, a single marker is usually suboptimal in terms of sensitivity and specificity. Panels of 2 or more antibodies usually are more effective and can improve diagnostic accuracy in fine-needle aspirates and paraffin-embedded tissue. Moreover, molecular profiling of tumors is a promising technologic approach that may unravel important novel biomarkers to be incorporated in the management of thyroid lesions.

References


